WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS

ON THE

EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

Some N-Nitroso Compounds

VOLUME 17

IARC, LYON

MAY 1978

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OF CHEMICALS TO HUMANS:

Some *N*-Nitroso Compounds

Volume 17

This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of the Carcinogenic Risk of Chemicals to Humans
which met in Lyon,
10-15 October 1977

May, 1978

IARC MONOGRAPHS

In 1971, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals.

The role of the monograph programme is to collect all available relevant experimental and epidemiological data about groups of chemicals to which humans are known to be exposed, to evaluate these data in terms of human risk with the help of international working groups of acknowledged experts in chemical carcinogenesis and related fields, and to publish and disseminate the conclusions of those working groups as a series of IARC Monographs.

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RISK OF CHEMICALS TO HUMANS:

SOME *N*-NITROSO COMPOUNDS

Lyon, 10-15 October 1977

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NOTE TO THE READER

The term 'carcinogenic risk' in the IARC Monograph series is taken to mean the probability that exposure to the chemical will lead to cancer in humans.

Inclusion of a chemical in the monographs does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that a chemical has not yet been evaluated in a monograph does not mean that it is not carcinogenic.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of a chemical for humans is encouraged to make this information available to the Unit of Chemical Carcinogenesis, International Agency for Research on Cancer, Lyon, France, in order that the chemical may be considered for reevaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Chemical Carcinogenesis, so that corrections can be reported in future volumes.

CONTENTS

IARC MONOGRAPH PROGRAMME ON THE EVALUATION OF THE
CARCINOGENIC RISK OF CHEMICALS TO HUMANS

PREAMBLE

BACKGROUND

In 1971, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans centred on the production of critically evaluated monographs on individual chemicals. Since 1972, the programme has undergone considerable expansion, primarily with the scientific collaboration and financial support of the US National Cancer Institute.

The criteria established in 1971 to evaluate the carcinogenic risk of chemicals to humans were adopted in essence by all the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monograph* series. In October 1977, a joint IARC/WHO *ad hoc* Working Group met to reevaluate these guiding criteria; this preamble reflects the results of their deliberations[1].

OBJECTIVE AND SCOPE

The objective of the monograph programme is to collect all available relevant experimental and epidemiological data about groups of chemicals to which humans are known to be exposed, to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields, and to publish and disseminate the conclusions of those working groups as a series of monographs.

The critical evaluations of experimental data given in these monographs are intended to assist national and international authorities in formulating decisions concerning preventive measures. The WHO publications on food additives[2], drugs[3], pesticides and contaminants[4] and occupational carcinogens[5] are particularly informative.

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Since the programme began in 1971, 17 volumes have been published[6-22] in the *IARC Monograph* series, and 380 separate chemicals have been evaluated (see cumulative index to the monographs, p. 353). Each volume is printed in 4000 copies and distributed *via* the World Health Organization (WHO) publications service (see inside covers for a listing of IARC publications and back outside cover for distribution and sales services).

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of environmental chemicals. The first users' survey, made in 1976, indicates that the monographs are consulted routinely by various agencies in 24 countries.

SELECTION OF CHEMICALS FOR MONOGRAPHS

The chemicals (natural and synthetic, mixtures and manufacturing processes) are selected for evaluation on the basis of two main criteria: (1) there is evidence of human exposure, and (2) there is some experimental evidence of carcinogenicity and/or there is some evidence or suspicion of a risk to humans. *Inclusion of a chemical in a volume does not imply that the chemical is carcinogenic, only that the published data have been examined. The evaluations must be consulted to ascertain the conclusions of the Working Group. Equally, the fact that a chemical has not appeared in a monograph does not mean that the chemical is not carcinogenic.*

The scientific literature is monitored for published data relevant to the monograph programme. Additionally, the IARC Survey of Chemicals Being Tested for Carcinogenicity[23-29] often indicates those chemicals that are to be scheduled for future meetings. The major aims of the survey are to prevent unnecessary duplication of research, to increase communication among scientists, and to make a census of chemicals that are being tested and of available research facilities.

When new, relevant information becomes available concerning a chemical(s) which has already been evaluated, or when new principles for evaluating carcinogenic risk receive acceptance, reevaluations may be made at subsequent meetings, and a monograph(s) may be revised and published.

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WORKING PROCEDURES

Approximately one year in advance of a working group meeting, a list of the substances to be considered is prepared by IARC in consultation with other experts. Subsequently, all relevant biological data are collected by IARC; in this context, US Public Health Service Publication No. 149[30-35] has been particularly valuable and has been used in conjunction with other recognized sources of information on chemical carcinogenesis. The major effort in the collection of data and the preparation of first drafts for the sections on chemical and physical properties, on production, use and occurrence and on analysis is made by SRI International under a separate contract with the US National Cancer Institute. Most of the data they provide on production, use and occurrence concern the United States and Japan; SRI and IARC try to supplement this information with that from other sources in Europe. Important bibliographical sources for mutagenicity and teratogenicity data are the Environmental Mutagen Informational Center and the Environmental Teratology Information Center, both located at the Oak Ridge National Laboratory, USA.

Six to nine months before the meeting, reprints of articles containing relevant biological data are sent to an expert(s), or are used by the IARC staff, for the preparation of first drafts of the monographs. These drafts are edited by IARC staff and are sent prior to the meeting to all participants of the Working Group for their comments. The Working Group then meets in Lyon for seven to eight days to discuss and finalize the texts of the monographs and to formulate the evaluations. After the meeting, the master copy of each monograph is verified by consulting the original literature, then edited and prepared for reproduction. The monographs appear in print within six months after adjournment of the Working Group meeting.

DATA FOR EVALUATIONS

With regard to biological data, generally only reports that have been published or accepted for publication are reviewed by the working groups. The monographs do not cite all of the literature on a particular chemical:

only those data considered by the Working Group to be relevant to the evaluation of the carcinogenic risk of the chemical to humans are included.

Anyone who is aware of data that have been published or are in press which are relevant to the evaluations of the carcinogenic risk to humans of chemicals for which monographs have appeared is urged to make them available to the Unit of Chemical Carcinogenesis, International Agency for Research on Cancer, Lyon, France.

THE WORKING GROUP

During a meeting the tasks of the Working Group are generally fivefold: (1) to confirm that all relevant published data are included; (2) to ensure that the summaries of data enable the reader to follow the reasoning of the committee; (3) to judge the significance of the experimental and epidemiological results; (4) to select and summarize the data on which to base an evaluation; and (5) to formulate an evaluation of the carcinogenic risk of the chemical.

Working Group participants who contributed to the consideration and evaluation of chemicals within a particular volume are listed, with their addresses, at the beginning of each publication (see p. 3). Each member serves as an individual scientist and not as a representative of any organization or government. In addition, observers are often invited from national and international agencies, organizations and industries.

GENERAL PRINCIPLES FOR EVALUATING THE CARCINOGENIC RISK OF CHEMICALS

The widely accepted meaning of the term 'chemical carcinogenesis', and that used in these monographs, is the induction by chemicals of neoplasms that are not usually observed, the earlier induction by chemicals of neoplasms that are usually observed, and/or the induction by chemicals of more neoplasms than are usually found, although fundamentally different mechanisms may be involved in these three phenomena. Etymologically, the term 'carcinogenesis' means the induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign tumours.

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Within the monographs, the words 'tumour' and neoplasm' are used inter-
changeably (In scientific literature the terms 'tumourigen', 'oncogen'
and 'blastomogen' have all been used synonymously with 'carcinogen',
although occasionally 'tumourigen' has been used specifically to denote
the induction of benign tumours).

The term 'carcinogenic risk' in this *IARC Monograph* series is taken
to mean the probability that exposure to the chemical will lead to cancer
in humans.

Experimental Evidence

Qualitative aspects

Both the interpretation and evaluation of a particular study as well
as the overall assessment of the carcinogenic activity of a chemical
involve several qualitatively important considerations, including:
(1) the experimental conditions under which the chemical was tested,
including route of administration and exposure, species, strain, sex, age,
etc.; (2) the consistency with which the chemical has been shown to be
carcinogenic, e.g., in how many species and at which tumour sites(s);
(3) the spectrum of neoplastic response, from benign neoplasia to multiple
malignant tumours (this consideration warrants special attention);
(4) the stage of tumour formation in which a chemical may be involved:
some chemicals act as complete carcinogens and have initiating and promot-
ing activity, while others are promoters only; and 5) the possible role
of modifying agents.

Many chemicals induce both benign and malignant tumours; few instan-
ces are recorded in which only benign neoplasms are induced by chemicals
that have been studied extensively. Benign tumours may represent a stage
in the evolution of a malignant neoplasm or they may be 'end-points'
which do not readily undergo transition to malignant neoplasms. If a
substance is found to induce only benign neoplasms in experimental animals,
the chemical should be suspected of being a carcinogen and requires further
investigation.

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Hormonal carcinogenesis

Hormonal carcinogenesis presents certain distinctive features: the chemicals involved occur both naturally and exogenously; in most instances, long exposure is required; tumours occur in the target issue in association with a stimulation of non-neoplastic growth, but in some cases, hormones promote the proliferation of tumour cells in a target organ. Hormones that occur in excessive amounts, hormone-mimetic agents and agents that cause hyperactivity or imbalance in the endocrine system may require evaluative methods comparable with those used to identify chemical carcinogens; particular emphasis must be laid on quantitative aspects and duration of exposure. Some chemical carcinogens have significant side effects on the endocrine system, which may also result in hormonal carcinogenesis. Synthetic hormones and anti-hormones can be expected to possess other pharmacological and toxicological actions in addition to those on the endocrine system, and in this respect they must be treated like any other chemical with regard to intrinsic carcinogenic potential.

Quantitative aspects

Dose-response studies are important in the evaluation of carcinogenesis: the confidence with which a carcinogenic effect can be established is strengthened by the observation of an increasing incidence of neoplasms with increasing exposure.

The assessment of carcinogenicity in animals is frequently complicated by recognized differences among the test animals (species, strain, sex, age), in route(s) of administration and in dose/duration of exposure; often, target organs at which a cancer occurs and its histological type may vary with these conditions. Nevertheless, indices of carcinogenic potency in particular experimental systems (for instance, the dose-rate required under continuous exposure to halve the probability of the animals remaining tumourless[36]) have been formulated in the hope that, at least among categories of fairly similar agents, such indices may be of some predictive value in other systems, including humans.

Chemical carcinogens differ widely in the dose required to produce a given level of tumour induction, although many of them share common

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biological properties which include metabolism to reactive (electro-philic[37-39]) intermediates capable of interacting with DNA. The reason for this variation in dose-response is not understood but may be due either to differences within a common metabolic process or to the operation of qualitatively distinct mechanisms.

Statistical analysis of animal studies

Tumours which would have arisen had an animal lived longer may not be observed because of the death of the animal from unrelated causes, and proper allowance must be made for this possibility. Various analytical techniques have been developed which use the assumption of independence of competing risks to allow for the effects of intercurrent mortality on the final numbers of tumour-bearing animals in particular treatment groups.

For externally visible tumours and for neoplasms that cause death, methods such as Kaplan-Meier (i.e., 'life-table', 'product-limit' or 'actuarial') estimates[36], with associated significance tests[40,41], are recommended.

For internal neoplasms which are discovered 'incidentally'[40] at autopsy but which did not cause the death of the host, different estimates[42] and significance tests[40,41] may be necessary for the unbiased study of the numbers of tumour-bearing animals.

All of these methods[36,40,41,42] can be used to analyse the numbers of animals bearing particular tumour types, but they do not distinguish between animals with one or many such tumours. In experiments which end at a particular fixed time with the simultaneous sacrifice of many animals, analysis of the total numbers of internal neoplasms per animal found at autopsy at the end of the experiment is straightforward. However, there are no adequate statistical methods for analysing the numbers of particular neoplasms that kill an animal host.

There are problems not only of differential survival but of differential toxicity, which may be manifested by unequal growth and weight gain in treated and control animals. These complexities should also be considered in the interpretation of data, or, better, in the experimental design.

Evidence of Carcinogenicity in Humans

Evidence of carcinogenicity in humans can be derived from three types
of study, the first two of which usually provide only suggestive evidence:
(1) reports concerning individual cancer patients (case reports), including
a history of exposure to the supposed carcinogenic agent;   (2) descriptive
epidemiological studies in which the incidence of cancer in human popula-
tions is found to vary (spatially or temporally) with exposure to the
agent);   and (3) analytical epidemiological studies (e.g., case-control
or cohort studies) in which individual exposure to the agent is found to
be associated with an increased risk of cancer.

An analytical study that shows a positive association between an
agent and a cancer may be interpreted as implying causality to a greater
or lesser extent, if the following criteria are met: (1) There is no
identifiable positive bias (By 'positive bias' is meant the operation of
factors in study design or execution which lead erroneously to a more
strongly positive association between an agent and disease than in fact
exists.   Examples of positive bias include, in case-control studies, more
nearly complete ascertainment of exposure to the agent in cases than in
controls and, in cohort studies, more nearly complete detection of cancer
in individuals exposed to the agent than in individuals not exposed).
(2) The possibility of positive confounding has been considered (By 'posi-
tive confounding' is meant a situation in which the relationship between
an agent and a disease is rendered more strongly positive than it truly
is as a result of an association between that agent and another agent
which either causes or prevents the disease.   An example of positive
confounding is the association between coffee consumption and lung cancer,
which results from their joint association with cigarette smoking).
(3) The association is unlikely to be due to chance alone.   (4) The asso-
ciation is strong.   (5) There is a dose-response relationship.

In some instances, a single epidemiological study may be strongly
indicative of a cause-effect relationship, however, the most convincing
evidence of causality comes when several independent studies done under
different circumstances result in 'positive' findings.

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Analytical epidemiological studies that show no association between an agent and a cancer ('negative' studies) should be interpreted according to criteria analogous to those listed above: (1) There is no identifiable negative bias. (2) The possibility of negative confounding has been considered. (3) The possible effects of misclassification of exposure or outcome have been considered.

In addition, it must be recognized that in any study there are confidence limits around the estimate of association or relative risk. In a study regarded as 'negative', the upper confidence limit may indicate a relative risk substantially greater than unity; in that case, the study excludes only relative risks that are above this upper limit. This usually means that a 'negative' study must be large to be convincing. Confidence in a 'negative' result is increased when several independent studies carried out under different circumstances are in agreement.

Finally, a 'negative' study may be considered to be relevant only to dose levels within or below the range of those observed in the study and is pertinent only if sufficient time has elapsed since first human exposure to the agent. Experience with human cancers of known etiology suggests that the period from first exposure to a chemical carcinogen to development of clinically observed cancer is usually measured in decades and may be in excess of 30 years.

Experimental Data Relevant to the Evaluation of Carcinogenic Risk to Humans

No adequate criteria are presently available to interpret experimental carcinogenicity data directly in terms of carcinogenic potential for humans. Nonetheless, utilizing data collected from appropriate tests in animals, positive extrapolations to possible human risk can reasonably be approximated.

Information compiled from the first 17 volumes of the *Monographs*[43-45] shows that of about 26 chemicals or manufacturing processes now generally accepted to cause cancer in humans, all but possibly two (arsenic and benzene) of those which have been tested appropriately produce cancer in at least one animal species. For several (aflatoxins, 4-aminobiphenyl, diethylstilboestrol, melphalan, mustard gas and vinyl chloride), evidence

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of carcinogenicity in experimental animals preceded evidence obtained from epidemiological studies or case reports.

In general, the evidence that a chemical produces tumours in experimental animals is of two degrees: (1) *sufficient evidence* of carcinogenicity is indicated by the production of malignant tumours; and (2) *limited evidence* of carcinogenicity reflects the qualitative and/or quantitative limitations of the experimental results.

For many of the chemicals evaluated in the first 17 volumes of the *IARC Monographs* for which there is *sufficient evidence* of carcinogenicity in animals, data relating to carcinogenicity for humans are either insufficient or nonexistent. In the absence of adequate data on humans, it is reasonable to regard for practical purposes such chemicals as if they were carcinogenic to humans.

Sufficient evidence of carcinogenicity is provided by experimental studies that show an increased incidence of malignant tumours: (a) in multiple species or strains, and/or (b) in multiple experiments (routes and/or doses), and/or (c) to an unusual degree (with regard to incidence, site, type and/or precocity of onset). Additional evidence may be provided by data concerning dose-response, mutagenicity or structure.

In the present state of knowledge, it would be difficult to define a predictable relationship between the dose (mg/kg bw/day) of a particular chemical required to produce cancer in test animals and the dose which would produce a similar incidence of cancer in humans. The available data suggest, however, that such a relationship may exist[46], at least for certain classes of carcinogenic chemicals. Data that provide *sufficient evidence* of carcinogenicity in test animals may therefore be used in an approximate quantitative evaluation of the human risk at some given exposure level, provided that the nature of the chemical concerned and the physiological, pharmacological and toxicological differences between the test animals and humans are taken into account. However, no acceptable methods are currently available for quantifying the possible errors in such a procedure, whether it is used to generalize between species or to extrapolate from high to low doses. The methodology for such quantitative extrapolation to humans requires further development.

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Evidence for the carcinogenicity of some chemicals in experimental animals may be <u>limited</u> for two reasons. Firstly, experimental data may be restricted to such a point that it is not possible to determine a causal relationship between administration of a chemical and the development of a particular lesion in the animals. Secondly, there are certain neoplasms, including lung tumours and hepatomas in mice, which have been considered of lesser significance than neoplasms occurring at other sites for the purpose of evaluating the carcinogenic risk of chemicals to humans. Such tumours occur spontaneously in high incidence in these animals, and their malignancy is often difficult to establish. An evaluation of the significance of these tumours following administration of a chemical is the responsibility of the particular Working Group preparing the individual monograph, and it has not been possible to set down rigid guidelines; the relevance of these tumours must be determined by considerations which include experimental design and completeness of reporting.

Some chemicals for which there is *limited evidence* of carcinogenicity in animals have also been studied in humans with, in general, inconclusive results. While such chemicals may indeed be carcinogenic to humans, more experimental and epidemiological investigation is required.

Hence, '*sufficient evidence*' of carcinogenicity and '*limited evidence*' of carcinogenicity do not indicate categories of chemicals: the inherent definitions of those terms indicate varying degrees of experimental evidence, which may change if and when new data on the chemicals become available. The main drawback to any rigid classification of chemicals with regard to their carcinogenic capacity is the as yet incomplete knowledge of the mechanism(s) of carcinogenesis.

In recent years, several short-term tests for the detection of potential carcinogens have been developed. When only inadequate experimental data are available, positive results in validated short-term tests (see p. 25) are an indication that the compound is a potential carcinogen and that it should be tested in animals for an assessment of its carcinogenicity. Negative results from short-term tests cannot be considered sufficient evidence to rule out carcinogenicity. Whether short-term tests will

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eventually attain a stature similar to that of long-term tests in predict-
ing carcinogenicity in humans will depend on further demonstrations of
consistency with long-term experiments and with data from humans.

EXPLANATORY NOTES ON THE MONOGRAPH CONTENTS

Chemical and Physical Data (Section 1)

The Chemical Abstracts Service Registry Number and the latest Chemical
Abstracts Primary Name (9th Collective Index) are recorded in section 1.
Other synonyms and trade names are given, but this list is often not
comprehensive. Further, some of the trade names are those of mixtures
in which the compound being evaluated is only one of the ingredients.

The structural and molecular formulae, molecular weight and chemical
and physical properties are given. The properties listed refer to the pure
substance, unless otherwise specified, and include, in particular, data that
might be relevant to carcinogenicity (for example, lipid solubility) and
those that concern identification. A separate description of the composition
of technical products includes available information on impurities and
formulated products.

Production, Use, Occurrence and Analysis (Section 2)

The purpose of section 2 is to provide indications of the extent of
past and present human exposure to this chemical.

Synthesis

Since cancer is a delayed toxic effect, the dates of first synthesis
and of first commercial production of the chemical are provided. In
addition, methods of synthesis used in past and present commercial produc-
tion are described. This information allows a reasonable estimate to be
made of the time before which no human exposure could have occurred.

Production

Since Europe, Japan and the United States are reasonably representative
industrialized areas of the world, most data on production, foreign trade
and uses are obtained from those countries. It should not, however, be

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inferred that those nations are the sole or even the major sources of
users of any individual chemical.

Production and foreign trade data are obtained from both governmental
and trade publications by chemical economists in the three geographical
areas. In some cases, separate production data on organic chemicals manu-
factured in the United States are not available because their publication
could disclose confidential information. In such cases, an indication of
the minimum quantity produced can be obtained from the number of companies
reporting commercial production. Each company is required to report on
individual chemicals if the sales value or the weight of the annual produc-
tion of a chemical exceeds a specified minimum level. These levels vary
for chemicals classified for different uses, e.g., medicinals, plastics;
however, the minimal annual sales value is between $1000 and $50,000 and
the minimal annual weight of production is between 450 and 22,700 kg.
Data on production in some European countries are obtained by means of
general questionnaires sent to companies thought to produce the compounds
being evaluated. Information from the completed questionnaires is compiled
by country, and the resulting estimates of production are included in the
individual monographs.

Use

Information on uses is meant to serve as a guide only and is not
complete. It is usually obtained from published data but is often comple-
mented by direct contact with manufacturers of the chemical. In the case
of drugs, mention of their therapeutic uses does not necessarily represent
current practice nor does it imply judgement as to their clinical efficacy.

Statements concerning regulations and standards (e.g., pesticide
registrations, maximum levels permitted in foods, occupational standards
and allowable limits) in specific countries are mentioned as examples only.
They may not reflect the most recent situation, since such legislation is
in a constant state of change; nor should it be taken to imply that other
countries do not have similar regulations.

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Occurrence

Information on the occurrence of a chemical in the environment is
obtained from published data, including that derived from the monitoring
and surveillance of levels of the chemical in occupation environments, air,
water, soil, foods and tissues of animals and humans. When available,
data on the generation, persistence and bioaccumulation of a chemical are
also included.

Analysis

The purpose of the section on analysis is to give the reader an indi-
cation, rather than a complete review, of methods cited in the literature.
No attempt is made to evaluate critically or to recommend any of the methods.

Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans
(Section 3)

In general, the data recorded in section 3 are summarized as given by
the author; however, certain shortcomings of reporting, of statistical
analysis or of experimental design are commented upon by the Working Group
in square brackets. The nature and extent of impurities/contaminants in
the chemicals being tested are given when available.

Carcinogenicity and related studies in animals

The monographs are not intended to cover all reported studies. Some
studies are purposely omitted (1) because they are inadequate, as judged
from previously described criteria[47-50] (e.g., too short a duration, too
few animals, poor survival); (2) because they only confirm findings that
have already been fully described; or (3) because they are judged irrele-
vant for the purpose of the evaluation. In certain cases, however, such
studies are mentioned briefly, particularly when the information is consi-
dered to be a useful supplement to other reports or when it is the only
data available. Their inclusion does not, however, imply acceptance of
the adequacy of their experimental design and/or of the analysis and
interpretation of their results.

Mention is made of all routes of administration by which the compound
has been adequately tested and of all species in which relevant tests have

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been done"[50]. In most cases, animal strains are given (general charac-
teristics of mouse strains have been reviewed[51]). Quantitative data are
given to indicate the order of magnitude of the effective carcinogenic
doses. In general, the doses and schedules are indicated as they appear
in the original paper; sometimes units have been converted for easier
comparison. Experiments on the carcinogenicity of known metabolites,
chemical precursors, analogues and derivatives, and experiments on factors
that modify the carcinogenic effect are also reported.

Other relevant biological data

Lethality data are given when available, and other data on toxicity
are included when considered relevant. The metabolic data are restricted
to studies that show the metabolic fate of the chemical in animals and
man, and comparisons of data from animals and humans are made when possible.
Information is also given on absorption, distribution, excretion and pla-
cental transfer.

Embryotoxicity and teratogenicity

Data on teratogenicity from studies in experimental animals and obser-
vations in humans are also included. There appears to be no necessary
causal relationship between teratogenicity[52] and carcinogenicity, but
chemicals often have both properties. Evidence of teratogenicity suggests
transplacental transfer, which is a prerequisite for transplacental carcino-
genesis.

Mutagenicity and other short-term tests

Data from indirect tests are also included. Since most of these tests
have the advantage of taking less time and being less expensive than
mammalian carcinogenicity studies, they are generally known as 'short-term'
tests. They comprise assay procedures which rely on the induction of
biological and biochemical effects in *in vivo* and/or *in vitro* systems.
The end-point of the majority of these tests is not the production of neo-
plasms in animals but changes at the molecular, cellular or multicellular
level: these include the induction of DNA damage and repair, mutagenesis
in bacteria and other organisms, transformation of mammalian cells in
culture, and other systems.

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The short-term tests are proposed for use (1) in predicting potential carcinogenicity in the absence of carcinogenicity data in animals, (2) as a contribution in deciding which chemicals should be tested in animals, (3) in identifying active fractions of complex mixtures containing carcino-ges, (4) for recognizing active metabolites of known carcinogens in human and/or animal body fluids and (5) to help elucidate mechanisms of carcino-genesis.

Although the theory that cancer is induced as a result of somatic mutation suggests that agents which damage DNA *in vivo* may be carcinogens, the precise relevance of short-term tests to the mechanism by which cancer is induced is not known. Predictions of potential carcinogenicity are currently based on correlations between responses in short-term tests and data from animal carcinogenicity and/or human epidemiological studies. This approach is limited because the number of chemicals known to be carcinogenic in humans is insufficient to provide a basis for validation, and most validation studies involve chemicals that have been evaluated for carcinogenicity only in animals. The selection of chemicals is in turn limited to those classes for which data on carcinogenicity are available. The results of validation studies could be strongly influenced by such selection of chemicals and by the proportion of carcinogens in the series of chemicals tested; this should be kept in mind when evaluating the predictivity of a particular test. The usefulness of any test is reflected by its ability to classify carcinogens and noncarcinogens, using the animal data as a standard; however, animal tests may not always provide a perfect standard. The attainable level of correlation between short-term tests and animal bioassays is still under investigation.

Since many chemicals require metabolism to an active form, test systems that do not take this into account may fail to detect certain potential carcinogens. The metabolic activation systems used in short-term tests (for example, the cell-free systems used in bacterial tests) are meant to simulate the intact human. Each has its advantages and limitations; thus, more confidence can be placed in the conclusions when negative or positive results for a chemical are confirmed in several such test systems. Defi-ciencies in metabolic competence may lead to misclassification of chemicals,

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which means that not all tests are suitable for assessing the potential carcinogenicity of all classes of compounds.

The present state of knowledge does not permit the selection of a specific test(s) as the most appropriate for identifying potential carcinogenicity. Before the results of a particular test can be considered to be fully acceptable for predicting potential carcinogenicity, certain criteria should be met: (1) the test should have been validated with respect to known animal carcinogens and found to have a high capacity for discriminating between carcinogens and noncarcinogens, and (2) when possible, a structurally related carcinogen(s) and noncarcinogen(s) should have been tested simultaneously with the chemical in question. The results should have been reproduced in different laboratories, and a prediction of carcinogenicity should have been confirmed in additional test systems. Confidence in positive results is increased if a mechanism of action can be deduced and if appropriate dose-response data are available. For optimum usefulness, data on purity must be given.

The short-term tests in current use that have been the most extensively validated are the *Salmonella typhimurium* plate-incorporation assay[53-57], the X-linked recessive lethal test in *Drosophila melanogaster*[58], unscheduled DNA synthesis[59] and *in vitro* transformation[57,60]. Each is compatible with current concepts of the possible mechanism(s) of carcinogenesis.

An adequate assessment of the genetic activity of a chemical depends on data from a wide range of test systems. The monographs include, therefore, data not only from those already mentioned, but also on the induction of point mutations in other systems[61-66], of structural[67] and numerical chromosome aberrations, including dominant lethal effects[68], of mitotic recombination in fungi[61] and of sister chromatid exchanges[69,70].

The existence of a correlation between quantitative aspects of mutagenic and carcinogenic activity has been suggested[4,68-74], but it is not sufficiently well established to allow general use.

Further information about mutagenicity and other short-term tests is given in references 71-77.

Case reports and epidemiological studies

Observations in humans are summarized in this section.

Summary of Data Reported and Evaluation (Section 4)

Section 4 summarizes the relevant data from animals and humans and gives the critical views of the Working Group on those data.

Experimental data

Data relevant to the evaluation of the carcinogenicity of a chemical in animals are summarized in this section. Results from validated mutagenicity and other short-term tests are reported if the Working Group considered the data to be relevant. Dose-response data are given when available. An assessment of the carcinogenicity of the chemical in animals is made on the basis of all of the available data.

The animal species mentioned are those in which the carcinogenicity of the substance was clearly demonstrated. The route of administration used in experimental animals that is similar to the possible human exposure is given particular mention. Tumour sites are also indicated. If the substance has produced tumours after prenatal exposure or in single-dose experiments, this is indicated.

Human data

Human exposure to the chemical is summarized, and the significance of data on production, use and occurrence and other relevant biological data is discussed. Case reports and epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are described. Adequate dose-response data are given when available. An assessment of the carcinogenicity of the chemical in humans is made on the basis of all of the available evidence.

Evaluation

This section comprises the overall evaluation by the Working Group of the carcinogenic risk of the chemical to humans. All of the data in the monograph, and particularly the summarized information on experimental and human data, are considered in order to make an evaluation. In addition, recommendations are made regarding areas in which further investigation is considered to be necessary.

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GENERAL REMARKS ON THE SUBSTANCES CONSIDERED

In this seventeenth volume of the *IARC Monograph* series, certain *N*-nitroso compounds are evaluated. Among these are a number of *N*-nitrosamines that have been tested for carcinogenicity and that have been shown to occur in the environment; the nitrosamides, *N*-nitroso-*N*-ethylurea and *N*-nitroso-*N*-methylurea; and the anticancer drug, streptozotocin, which is related structurally to the latter nitrosamide. Monographs on *N*-nitrosodiethylamine, *N*-nitrosodimethylamine, *N*-nitroso-*N*-ethylurea and *N*-nitroso-*N*-methylurea appeared in volume 1 of the *IARC Monographs*, and monographs on *N*-nitrosodi-*n*-butylamine and streptozotocin were published in volume 4; new data on these compounds have been included in the present volume.

In addition, a monograph on polychlorinated biphenyls, published in volume 7 of the *IARC Monographs*, was re-examined and updated to include recent observations on the effects of this class of chemicals on humans. The resulting monograph will be combined with a future one on polybrominated biphenyls, and these are to be published as a separate volume.

Following the observation in animals of the toxic (Barnes & Magee, 1954) and carcinogenic effects of *N*-nitrosodimethylamine (Magee & Barnes, 1956), studies were made of the biological activities of a variety of *N*-nitroso compounds. The hepatotoxic effect of *N*-nitrosodimethylamine in humans was reported in 1937 by Freund (1937), who described clinical and autopsy findings in two chemists accidentally poisoned with this compound. The carcinogenicity of several *N*-nitroso compounds was extensively investigated and reported by Druckrey *et al.* (1967) and was reviewed by Magee & Barnes (1967). More recently, reviews on their toxicity (Magee & Swann, 1969), carcinogenicity and metabolism (Magee *et al.*, 1975, 1976), teratogenicity (Druckrey, 1973a,b) and mutagenicity (Montesano & Bartsch, 1976; Neale, 1976) have also appeared. The carcinogenicity studies have demonstrated that these *N*-nitroso compounds have a high degree of specificity in inducing tumours in various species and among target organs within the same species. This group of carcinogens has provided a unique research tool for elucidating the possible mechanism(s) of cancer induction by chemical substances at cellular and molecular levels (Magee *et al.*, 1976; Pegg, 1977).

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The observation of severe liver disease in sheep fed a diet containing fishmeal preserved with nitrite (Koppang *et al.*, 1964) and the identification of *N*-nitrosodimethylamine as the toxic principle in such products (Ender *et al.*, 1964; Sakshaug *et al.*, 1965) led to widespread efforts to evaluate levels of *N*-nitroso compounds in food destined for human and animal consumption.

Humans may be exposed to *N*-nitroso compounds in several ways: formation in the environment and subsequent absorption from food, water, air or industrial and consumer products; formation in the human body from precursors ingested separately in food, water or air; from the consumption or smoking of tobacco; and from naturally occurring compounds.

It has been known since 1865 that the reaction of dimethylamine hydrochloride with sodium nitrite at an acidic pH yields *N*-nitrosodimethylamine (Fridman *et al.*, 1971). It has been suggested (Druckrey *et al.*, 1963; Lijinsky & Epstein, 1970; Sander, 1967) that this reaction could occur in the acid conditions of the mammalian stomach between ingested nitrite and amines, and there is now substantial evidence that *N*-nitrosamines can be formed in this way. The phenomenon was first demonstrated by Sander *et al.* (1968).

Nitrite and nitrate are added to some foods, particularly meats and fish, as processing aids and as agents to prevent formation of botulinum toxin. White (1975) estimated that about 80% of the total nitrate ingested daily (106 mg) originates from vegetables, and about 70% of nitrite that enters the stomach (12.8 mg) comes from the saliva. Regulations exist in many countries to control the addition of nitrate and nitrite to food products. In order to prevent the possible formation of *N*-nitrosamines from, for example, mixtures of spices with nitrites and/or nitrates used in commercial meat and poultry curing (known as curing premixes), the US Food and Drug Administration has made a regulation that allows the use of these premixes only if the nitrites and/or nitrates are packaged separately from the spices with instructions on the label that they are not to be combined until just prior to use (US Food and Drug Administration, 1973). Nitrate reduction by bacteria could also lead to the formation of *N*-nitros-

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amines either *in vivo* (Hawksworth & Hill, 1971) or in the environment
(Ayanaba *et al.*, 1973).

Little is known about the metabolism of nitrate in man. Nitrite is
formed in the oral cavity by bacterial reduction of nitrate, and the normal
concentration range in individuals who do not consume large amounts of
nitrate is 6-10 mg/l of saliva (Tannenbaum *et al.*, 1974). When foods that
contain nitrate, such as certain root and leafy vegetables, are consumed,
salivary nitrate and nitrite levels are elevated to hundreds of mg/l
(Spiegelhalder *et al.*, 1976; Tannenbaum *et al.*, 1976). Under normal
conditions, nitrite arises in the stomach from the swallowing of saliva
(Klein *et al.*, 1978), but bacterial reduction of nitrate may continue in
the stomachs of individuals who have low gastric acidity or anacidity;
low gastric acidity has been related to the etiology of gastric cancer
(Cuello *et al.*, 1976).

Nitrosatable substances that occur in the environment include secon-
dary and tertiary amines, quaternary ammonium compounds, ureas, carbamates
and guanidines. Some of these compounds are found ubiquitously in nature;
nitrosation of a number of agricultural chemicals has been shown to occur
(Eisenbrand *et al.*, 1974; Elespuru & Lijinsky, 1973; Preussmann, 1975;
Sen *et al.*, 1974), and various drugs carry nitrosatable alkyl amino or
amido groups (Lijinksy, 1974; Lijinsky & Greenblatt, 1972). The chemical
kinetics of nitrosation have been reviewed recently by Mirvish (1975).

The drug amidopyrine can react rapidly with traces of nitrosating
agents (even gaseous nitrogen oxides), resulting in the formation of
N-nitrosodimethylamine; a wide variety of commercial drugs containing
amidopyrine have been found to have traces of this carcinogen (Eisenbrand
et al., 1978).

Evidence that *N*-nitroso compounds are formed in the body from amine
precursors is based on: (a) their detection in gastric juices *in vitro*
and in the mammalian or human stomach *in vivo*; and (b) the observation
of acute toxic and carcinogenic effects as well as damage to cellular
macromolecules after simultaneous administration of nitrite and various
amines and amides (Magee *et al.*, 1976; Preussmann, 1975). Recently,

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N-nitrosodiethyl- and -dimethylamines have been detected in human blood after the consumption of a meal consisting of normal dietary items (Fine *et al.*, 1977a).

The amount of N-nitroso compounds formed by nitrosation is affected by many factors, such as basicity of the amine, pH and substrate concentration. Nucleophilic ions such as thiocyanate increase the rate of N-nitrosamine formation (Boyland *et al.*, 1971; Fan & Tannenbaum, 1973). C-Nitrosophenols, the product of the nitrosation of phenols, catalyse N-nitrosamine formation (Davies *et al.*, 1978); other naturally occurring phenols may either inhibit or promote formation under different conditions (Walker *et al.*, 1975). Nitrosation may be promoted in certain environments by the presence of bacteria (Archer *et al.*, 1978; Klubes *et al.*, 1972). Ascorbic acid (Mirvish *et al.*, 1972) and α-tocopherol (Mergens *et al.*, 1978) inhibit the formation of N-nitroso compounds.

Other, as yet unexplored possibilities for the formation of N-nitroso compounds are: transnitrosation reactions (Singer *et al.*, 1978); nitrosation in organic solutions of strongly basic amines even by inorganic nitrite (Angeles *et al.*, 1978); and nitrosation even under strongly alkaline conditions with nitrogen oxides (Challis *et al.*, 1978).

Human exposure to N-nitroso compounds can also occur from contact with exogenously formed N-nitroso compounds. The occurrence of nitrosamines in foodstuffs has been reviewed recently (Crosby, 1976; Crosby & Sawyer, 1976). Significant levels of N-nitrosamines have also been found in cosmetics (Fan *et al.*, 1977a).

The possible formation of N-nitrosamines in cigarette smoke was originally suggested by Druckrey & Preussmann (1962), since tobacco contains several nitrosatable amines and nitrosating agents. Subsequently, nitrosamines were found in tobacco and tobacco smoke (Brunnemann & Hoffmann, 1978; Hoffmann *et al.*, 1974, 1976) and in indoor atmospheres under conditions of excessive tobacco smoking (Brunnemann & Hoffmann, 1978).

The occupational hazards associated with the use of N-nitroso compounds in industry have been outlined by Magee (1972). Some are used as organic accelerators and antioxidants in the production of rubber (Boyland *et al.*,

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1968). *N*-Nitrosodimethylamine was detected as a pollutant in the air of a factory producing dimethylamine; its concentration correlated with the concentrations of nitrogen dioxide and secondary amine in the air (Bretschneider & Matz, 1974, 1976). *N*-Nitrosodimethylamine and three unidentified *N*-nitroso compounds were also detected in samples of air in the vicinity of a plant manufacturing 1,1-dimethylhydrazine, a rocket propellant of which *N*-nitrosodimethylamine is a precursor, and in the vicinity of a factory manufacturing amines (Fine *et al.*, 1976a,b,c). Another source of human exposure to *N*-nitroso compounds may be the nitrite salts used as corrosion inhibitors in packaging materials (Archer & Wishnok, 1976), in synthetic cutting fluids (Fan *et al.*, 1977b; Rappe & Zingmark, 1978; Zingmark & Rappe, 1977) and in some widely used herbicides (Fine *et al.*, 1977b) (see also monographs on *N*-nitrosodi-*n*-butylamine, *N*-nitroso-dimethylamine and *N*-nitrosodiethanolamine).

There is considerable evidence that nitrosation reactions can occur in soils, organic waste or water in areas where industrial or other waste discharges contain large amounts of amines (Ayanaba *et al.*, 1973; Tate & Alexander, 1975).

The relevance of these traces of *N*-nitroso compounds to a possible risk to humans has yet to be established. However, a large variety of animal species and organs are susceptible to their carcinogenic action, and samples of human liver and lung are capable of forming alkylating (Harris *et al.*, 1977; Montesano & Magee, 1970) and mutagenic (Bartsch *et al.*, 1976; Czygan *et al.*, 1973) metabolites. The exceedingly difficult question of whether a proportion of human cancer or transmissible genetic damage could be attributed to these groups of carcinogens has not so far been answered, although some studies have indirectly examined the possible role of *N*-nitrosamines in the etiology of a number of human cancers (Correa *et al.*, 1975; Cuello *et al.*, 1976; Hill *et al.*, 1973; Joint Iran-IARC Study Group, 1977; Zaldívar & Wetterstrand, 1975).

At present, however, it may be difficult, if not impossible, to demonstrate in the general population a cause-effect relationship between exposure to low levels of *N*-nitrosamines and the incidence of certain human

cancers, although the existence of such a correlation remains a working
hypothesis.

Most of the chemical and physical properties of the nitrosamines
described in these monographs were taken from Druckrey *et al.* (1967).
The principal techniques employed for the analysis of volatile *N*-nitros-
amines have been described in a recent publication (Preussmann *et al.*,
1978). The relative merits of high- and low-resolution mass spectrometry
are discussed, since use of mass spectrometry as a confirmatory technique
is particularly important. In this respect, those data reported in the
section on occurrence which have not been confirmed by this technique have
been marked with a footnote. This does not necessarily imply that the data
are unreliable, but, in assessing the significance of such data, emphasis
should be placed on those that have been confirmed by mass spectrometry.
In certain instances, *N*-nitroso compounds may be formed as artefacts
during an analytical procedure; in these cases mass spectrometry adds
little to the value of the data (Fine *et al.*, 1977b).

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N-NITROSODIETHYLAMINE

This substance was considered previously by an IARC Working Group,
in December 1971 (IARC, 1972). Since that time new data have become
available, and these have been incorporated into the monograph and taken
into account in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 55-18-5

Chem. Abstr. Name: *N*-Ethyl-*N*-nitroso-ethanamine

DEN; DENA; *N,N*-diethylnitrosamine; diethylnitrosamine;
nitrosodiethylamine; NDEA

1.2 Structural and molecular formulae and weight

$$O=N-N\begin{array}{l} CH_2-CH_3 \\ CH_2-CH_3 \end{array}$$
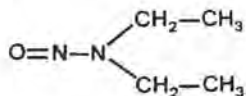
$$C_4H_{10}N_2O \qquad Mol.\ wt:\ 102.1$$

1.3 Chemical and physical properties of the pure substance

(a) Description: Yellow, volatile liquid

(b) Boiling-point: $177^{O}C$ (760 mm); $64-65^{O}C$ (17 mm)   (Druckrey
et al., 1967)

(c) Density: d_4^{20} 0.9422

(d) Refractive index: n_D^{20} 1.4386

(e) Spectroscopy data: λ_{max} 230 and 340 nm (E_1^1 = 726.7 and 8.3)
in water (Druckrey *et al.*, 1967); mass spectroscopy data are
given by Pensabene *et al.* (1972) and Rainey *et al.* (1978).

(f) Solubility: Soluble in water (approximately 10%) (Druckrey
et al., 1967) and in organic solvents and lipids

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(g) Volatility: Can be steam-distilled quantitatively (Eisenbrand *et al.*, 1970)

(h) Stability: Stable at room temperature for more than 14 days in neutral or alkaline aqueous solutions in the dark (Druckrey *et al.*, 1967); slightly less stable in acidic solutions; light-sensitive, especially to ultra-violet light

(i) Reactivity: Strong oxidants (peracids) oxidize it to the corresponding nitramine; can be reduced to the corresponding hydrazine and/or amine; relatively resistant to hydrolysis but can easily be split by hydrogen bromide in acetic acid (Eisenbrand & Preussmann, 1970). Photochemically reactive (Fridman *et al.*, 1971). A description of the preparation of various derivatives is available (Preussmann *et al.*, 1978).

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

For background information on this section, see preamble, p. 22.

(a) Production

N-Nitrosodiethylamine (NDEA) was first prepared in 1863 by Geuther & Kreutzhage (Prager *et al.*, 1922). It can be made by the reaction of diethylamine and nitrous acid (Druckrey *et al.*, 1967).

Although NDEA is available in small quantities for research purposes, no evidence was found that it has been manufactured commercially.

(b) Use

No data were available to the Working Group.

2.2 Occurrence

(a) Air

When the air of a number of major US cities was examined for N-nitrosamines, NDEA (200 mg/m^3)[1] was found only in one area of Baltimore (Fine *et al.*, 1976a,b). Levels of <10 ng/m^3[1] have been found in the smoking compartment of a train (Brunnemann & Hoffman, 1978).

(b) Tobacco smoke

McCormick *et al.* (1973) reported NDEA in tobacco smoke condensate at a level of 1-28 ng/cigarette. The mainstream smoke of a blended cigarette without filter tip (85 mm; 0.9% nitrate; 2.05% nicotine) was reported to contain <5 ng NDEA/cigarette (Hoffmann *et al.*, 1974). Klimsch *et al.* (1976) reported trace levels in 9 types of tobacco smoke, and 1 ng/cigarette in one sample. Up to 8.3 ng/cigarette were found in the mainstream smoke of 19 commercial and experimental cigarettes; far greater concentrations (8-73 ng/cigarette) were found in sidestream smoke (Brunnemann & Hoffmann, 1978; Brunnemann *et al.*, 1977).

(c) Water

NDEA was found at the 0.010 µg/l[1] level in high-nitrate well-water used for drinking (Fine, 1978). Fiddler *et al.* (1977) reported 0.33 and 0.83 µg/l NDEA in deionized water. Levels of 0.07 and 0.24 µg/l[1] were found in the waste-water from 2/19 chemical plants (Cohen & Bachman, 1978).

(d) Food and feed

Cheese: NDEA was detected in a sample of Cheddar cheese in trace amounts (>0.5 µg/kg but <1.0 µg/kg) and in Cheshire cheese at a level of 1.5 µg/kg[1] (Alliston *et al.*, 1972). It was found in 1/16 cheese samples at a level of 20-30 µg/kg[1] (Cerutti *et al.*, 1975). Sen *et al.* (1978) reported 2-20 µg/kg NDEA in 10/63 cheeses. Eisenbrand *et al.* (1978) found 3 µg/kg in 1/173 cheeses.

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[1]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).

<u>Vegetables and vegetable oils</u>: NDEA has been detected in beans at a level of 0.2 µg/kg[1] (Roach, 1972), and in five samples of freshly expressed soya bean oil at a level of 4 µg/kg[1] (Hedler & Marquardt, 1974).

<u>Cereal products</u>: In earlier work using inadequate methodology, conflicting reports were made about the presence of NDEA in cereal products. Hedler & Marquardt (1968), Heyns & Röper (1974), Kroeller (1967), Marquardt & Hedler (1966), Roach (1972), Sen *et al.* (1969), Thewlis (1967) and studies in Iran (Joint Iran-IARC Study Group, 1977) found no evidence of NDEA in such products.

<u>Fish</u>: NDEA has been found at levels of less than 1 µg/kg in spotted catfish, cod, coalfish, greater silver smelt, grenadier, herring, pollock, redfish and torsk; haddock was found to contain up to 4 µg/kg (Telling *et al.*, 1974). In another study, cod was found to contain up to 50 µg/kg[1] in salted samples, 39 µg/kg[1] in samples in tomato sauce, 1 µg/kg[1] in frozen samples and none in smoked samples. The same study reported that dwarf herring contained 147 µg/kg[1] in baked samples, 2 µg/kg[1] in fresh samples and 1 µg/kg[1] in smoked samples. Herring was found to contain 108 µg/kg[1] in pickled samples, 27 µg/kg[1] in samples in tomato sauce and 4 µg/kg[1] in smoked samples (Kann *et al.*, 1976). Earlier studies reported a level of 1.5 µg/kg[1] in both uncooked and fried stale cod (Alliston *et al.*, 1972) and traces[1] of NDEA in samples of pickled but not frozen herring (Sen *et al.*, 1969). Levels of 1.2-21.0 mg/kg[1] have been reported in Cantonese salt-dried fish (Fong & Walsh, 1971).

NDEA has also been detected[1] in salted salmon roe which contained sodium nitrite (Sakai & Tanimura, 1971). Iyengar *et al.* (1976) found 4-14 µg/kg NDEA in halibut, salmon, cod, sole, ocean perch and scallops only after frying or baking.

<u>Meat products</u>: A maximum of 40 µg/kg[1] NDEA was reported in cured meats (Freimuth & Glaeser, 1970). Less than 1 µg/kg was detected in 1/24 samples of fried bacon, and 1-4 µg/kg were found in a sample of chopped

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[1]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).

pork (Crosby *et al.*, 1972). In the UK, 1.5 µg/kg[1] NDEA was found in fried
back bacon and in uncooked pig's liver (Alliston *et al.*, 1972). Levels of
2-25 µg/kg were found in 11/298 samples (Sen *et al.*, 1974) and in 9/80
samples of various meat products (Panalaks *et al.*, 1974). Trace amounts[1]
of NDEA were reported in samples of tea sausage, smoked 'Krekovi' sausage
and smoked 'Servelaad' sausage (Kann *et al.*, 1976). Samples of uncooked
bacon were found to contain 0.3 µg/kg, and cooked bacon samples contained
2.0 µg/kg[1] (Fine & Rounbehler, 1976).

Sen *et al.* (1976) found 10 µg/kg in pepperoni, 3-10 µg/kg in bologna,
7 µg/kg in wieners, 10 µg/kg in mock chicken, 16 µg/kg in meatloaf and
5 µg/kg in ham sausage. Groenen *et al.* (1976) found 7 and 91 µg/kg in
two samples of smoked meat and 4-43 µg/kg in fried and unfried bacon.
Stephany *et al.* (1976) found a mean of 0.1-0.6 µg/kg in meat products,
with up to 2.8 µg/kg in cooked and smoked ham; and Groenen *et al.* (1977)
found 0.1-0.8 µg/kg in 11/47 samples of processed meats.

Havery *et al.* (1978) and Eisenbrand *et al.* (1978) found no evidence
of NDEA in a large number of meat products. Gough (1978) found 0.05 µg/kg[1]
in 1/13 prepared meals, <0.03 µg/kg[1] in 3/36 pastry foods and up to 0.2
µg/kg[1] in cured meats.

Feed: A level of 1 µg/kg NDEA was found in 4/6 samples of fishmeal
(Mirna *et al.*, 1976), and 28 and 36 µg/kg were found in 2/16 samples
(Juszkiewicz & Kowalski, 1976). Kann *et al.* (1978) reported <1.0 µg/kg
in 3/46 samples of animal feed. Levels of 0-17 µg/kg have been found in
experimental animal feed (IARC, 1977a).

(e) Alcoholic beverages

NDEA has been detected in a number of alcoholic beverages, including
apple brandy, ciders, cognac, armagnac, rum and whiskey. The average
level was 0.1 µg/kg (IARC, 1977b).

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[1]These results were not confirmed by mass spectroscopy (see also
'General Remarks on the Substances Considered', p. 40).

(f) *In vivo*

NDEA was present in human blood both before and after the ingestion of a meal: the level in the blood increased from 0.09 µl/l[1] before to 0.46 µg/l[1] following the meal (Fine *et al.*, 1977). Lakritz *et al.* (1978) reported the presence of 5–30 µg/kg NDEA in the stomachs of 4/35 fasting patients [No adequate precautions were taken to prevent nitrosamine formation during storage of the samples].

2.3 Analysis

An IARC Manual gives selected methods for the analysis of volatile *N*-nitrosamines, including NDEA (Preussmann *et al.*, 1978).

On 29 September 1977, the US Environmental Protection Agency issued a notice requiring all registrants of pesticide products that are potentially contaminated with *N*-nitroso compounds to analyse commercial samples which have been stored for at least 18 days to determine the extent of contamination. This notice prescribes in general terms the types of analytical methods to be used for volatile *N*-nitroso compounds (e.g., gas chromatography plus mass spectrometry or thermal energy analysis, and others) as well as for nonvolatile *N*-nitroso compounds (e.g., high-pressure liquid chromatography plus ultra-violet spectroscopy). Confirmation of positive results by gas chromatography and mass spectrometry or by valid independent methods is required when possible (US Environmental Protection Agency, 1977).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Man

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Several studies on different strains have demonstrated the carcinogenicity of *N*-nitrosodiethylamine (NDEA) in this species. In the

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[1]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).

liver, mainly haemangioendotheliomas (Schmähl & Thomas, 1965a; Schmähl
et al., 1963a) and adenomas (Takayama & Oota, 1965), but also hepatomas
(Clapp & Craig, 1967; Clapp et al., 1970; Schmähl et al., 1963a), were
produced. Squamous-cell carcinomas of the oesophagus and forestomach
were also found (Clapp & Craig, 1967; Clapp et al., 1970, 1971;
Shvemberger, 1965; Takayama & Oota, 1965). An increased incidence of
lung adenomas has also been observed (Clapp & Craig, 1967; Clapp et al.,
1970; Mirvish & Kaufman, 1970). The site and histological type of the
tumours depended to a certain extent on the mouse strain used (Clapp
et al., 1971). Tumour frequency was usually very high, approaching in
many cases 100%, in mice given a dosage of 2-13 mg/kg bw/day. Some indi-
cation of a dose-response relationship has been reported by Clapp et al.
(1970) and Schmähl & Thomas (1965a).

It was reported in an abstract that single i.g. doses of 60 mg/kg bw
NDEA produced 7/24 hepatomas and 7/24 lung adenomas in BTO mice; no sig-
nificant increase was seen in C57BL/60 mice (Akamatsu, 1975).

Rat: Following the first report of liver carcinogenesis by NDEA in
rats (Schmähl et al., 1960), many laboratories confirmed and enlarged
this finding using different strains and conditions (Grundmann & Sieburg,
1962; Lacassagne et al., 1967; Reid et al., 1963; Takayama et al.,
1975; Thomas, 1961). In most cases, hepatocellular tumours have been
observed, often with lung metastases; in some cases, cholangiomas have
also been described (Argus & Hoch-Ligeti, 1961; Hoch-Ligeti et al., 1964).
In lifetime feeding studies with daily doses of between 1 and 10 mg/kg bw,
tumour yields approaching 100% have been found. While most investigations
have shown no sex difference, Reuber & Lee (1968) reported an increased
sensitivity in young females. The same authors also reported that four-
week-old animals were more sensitive than older animals. Feeding for only
82 days increased the latent period and reduced the tumour yield as compared
with lifetime feeding (Rajewsky et al., 1966). With lower daily dosages
(0.15-0.6 mg/kg bw), squamous-cell carcinomas of the oesophagus were
obtained in addition to liver tumours (Druckrey et al., 1963a). A single
dose of 280 mg/kg bw induced liver and kidney tumours, and 4 weekly doses

of 25 or 35 mg/kg bw induced tumours of the liver, the oesophagus and the kidney (Druckrey *et al.*, 1963b, 1964).

In a dose-response study (Table 1), the mean total dose administered in drinking-water until appearance of the first tumour ranged from 56-965 mg/kg bw, and the induction time for tumours between 68 and 750 days. All dosages higher than 0.15 mg/kg bw/day gave a tumour yield of 100%; 0.15 mg/kg bw/day gave a tumour yield of 27/30. At a dose of 0.075 mg/kg bw/day, 20 rats lived for more than 600 days (40/60 died from pneumonia); at 750 days, 16 were dead and 1 animal had a hepatoma, 3 had multiple papillomas of the oesophagus, and 3 had a squamous-cell carcinoma of the nasal cavity. The four animals given this dose level that lived at least until 940 days of age (850 days of the experiment) all had tumours: 3 had hepatomas (one of these also had a jaw carcinoma) and one had a hepatocellular carcinoma; 2 sarcomas were seen also (Druckrey *et al.*, 1963a).

Liver tumours, including 13 hepatocellular carcinomas, 9 haemangio-sarcomas and 1 unclassified blastoma, were induced in 23/25 16-week-old male Wistar rats by daily administration of 1 mg NDEA in the drinking-water, up to a total dose of 134 mg/animal (Hadjiolov, 1972).

Carcinomas of the oesophagus were induced in 9/14 male and 5/14 female 12-week-old Buffalo rats after feeding 0.0114% NDEA in the diet for 26 weeks. Animals survived an average of 28 weeks (Reuber, 1975).

It was reported in an abstract that nasal and other tumours (unspecified) occurred after the tenth month of life in the offspring of a female rat given NDEA during nursing (route unspecified); the mother developed a kidney tumour and died after 9 months (Schoental & Appleby, 1973).

Hamster: The i.g. administration of 0.4 ml of a dilute aqueous solution of NDEA (1:250) twice weekly induced tumours of the trachea and/or lung in 37/68 Syrian golden hamsters (Dontenwill & Mohr, 1961; Dontenwill *et al.*, 1962). In another study, malignant liver-cell tumours and tumours of the nasal cavity and bronchi were also induced (Herrold & Dunham, 1963).

Tumours of the forestomach and oesophagus developed in all of 20 male and 20 female inbred Chinese hamsters that received 40 mg/l NDEA in

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Table 1[a]

N-Nitrosodiethylamine dose-response

Daily dose mg/kg bw	Number of animals alive at appearance of first malignant liver tumour	Number of animals with malignant tumours of the liver and/ or oesophagus	Number of malignant liver tumours	Number of lung metastases	Number of oesophageal tumours	
					Papillomas	Carcinomas
14.2	5	5	5			
9.6	25	25	25	4		
4.8	25	25	25	4		
2.4	34	34	34	7		
1.2	36	36	36	10		
0.6	49	49	49	8	4	5
0.3	67	62	4	4	7	23
0.15	30	27	18	2	13	9
0.075	4	1	1		3	

[a]From Druckrey et al., 1963a

drinking-water for 17-26 weeks. Squamous-cell carcinomas accounted for
23% of the stomach tumours and for 15% of those of the oesophagus. Hepato-
cellular carcinomas also occurred in 5 animals (Baker *et al.*, 1974).

Guinea-pig: NDEA administered in the drinking-water as 5 mg/kg
bw/day induced hepatocellular carcinomas and liver adenocarcinomas, some
metastasizing into the lungs, in all of 11 treated animals. The median
total dose was 1200 mg/kg bw (Druckrey & Steinhoff, 1962). With a dose
of 3 mg/kg bw/day, 7/8 animals had liver tumours (Thomas & Schmähl, 1963).
In another study, hepatocellular carcinomas were again the main tumour
type induced in 14/15 animals that lived more than 16 weeks (Argus &
Hoch-Ligeti, 1963). In a dose-response study, NDEA was given in the
drinking-water for periods of 4, 8, 12 and 24 weeks; with an average
daily intake of 1.2 mg/animal (total dose, <75 mg/animal), no tumours
were observed after one year. With a higher daily intake, treatment for
12 weeks gave a 21% tumour yield and treatment for 24 weeks a 100% yield
(Arcos *et al.*, 1969).

Rabbit: Daily doses of 3.4 mg/kg bw NDEA given continuously in the
drinking-water induced liver-cell carcinomas in 2/2 treated rabbits
(Schmähl & Thomas, 1965b). In another experiment, all 13 animals that
received NDEA continuously in the drinking-water (0.042 g/l) on 6 days a
week died with metastasizing hepatic carcinomas; one animal had an adeno-
carcinoma of the lung (Rapp *et al.*, 1965).

Dog: Primary hepatic neoplasms of various types were induced in 14/14
adult male mongrel dogs given 50, 100 or 500 mg/l NDEA in the drinking-water
for 2-50 weeks; these consisted of 3 fibromas, 4 leiomyomas, 1 haemangioma,
10 haemangioendotheliomas, 4 fibrosarcomas, 2 leiomyosarcomas, 1 hepato-
cellular carcinoma, 1 cholangiocarcinoma and 1 undifferentiated-cell car-
cinoma. Six dogs developed squamous-cell carcinomas of the nasal cavity
(Hirao *et al.*, 1974).

Pig: Various tumours of the liver, 1 adenoma of the kidney and 1
squamous-cell carcinoma of the ethmoid were induced by daily doses of
4.4 mg/kg bw NDEA to 4 pigs (Schmähl *et al.*, 1967). In another study, 2
pigs treated with 1.5 mg/kg bw for 11 months and then 3 mg/kg bw until

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death developed a hepatoma and a kidney adenoma, respectively (total doses, 750 mg/kg bw for the pig that died after 470 days and 1090 mg/kg bw for the one that died after 594 days) (Schmähl *et al.*, 1969).

A group of 6 Göttingen mini-pigs received doses of 0.4 mg/kg bw NDEA in aqueous solution 5 times/week for 42 weeks/year. After 5 years, 3 long-term survivors died (total dose, 420 mg/kg bw), and 2 others were killed. All 5 mini-pigs had a hepatocellular adenoma. Four animals had hepato-cellular carcinomas; one of these animals also had a Kupffer-cell sarcoma, and in another metastases of the hepatocellular carcinoma were found in the lung. In addition, 1 renal carcinoma and 1 brain tumour were observed (Graw & Berg, 1977).

Monkey: It was reported in an abstract that after oral administration of NDEA, beginning 12 hours after birth, at a dosage varying from 2-30 mg/kg bw/day, hepatocellular carcinomas were induced in 3/15 rhesus and cynomolgus monkeys (O'Gara & Kelly, 1965). Hepatocellular carcinomas were induced in 6/15 rhesus and cebus monkeys treated with various doses of NDEA for more than a year; treatment of newborn or young animals was with 2 mg/kg bw, and the dose was gradually increased up to 30 mg/kg bw. The cumulative oral dosage ranged from 6-26 g/monkey, and the induction time varied between 14 and 24 months (Kelly *et al.*, 1966).

(b) Skin application

Mouse: Twice-weekly skin application of two drops of a 0.2% solution of NDEA in acetone for 10 months induced squamous-cell carcinomas of the nasal cavity in 17/24 animals; no local skin tumours were observed (Hoffmann & Graffi, 1964a). Daily treatment with three drops of 0.2% solution, or twice-weekly treatment with two drops, induced squamous-cell carcinomas of the nasal cavity in almost all treated animals after appli-cation of more than 8 mg/animal (Hoffmann & Graffi, 1964b).

Hamster: In 6/8 Syrian hamsters, skin painting with NDEA in water produced epithelial papillomas of the nasal cavity, but no skin tumours (Herrold, 1964a,b).

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(c)   Inhalation and/or intratracheal administration

Rat: Spray inhalation of a dilute aqueous solution of NDEA (1:250)
for 4 months produced liver carcinomas in 8/17 animals, but no lung
tumours (Dontenwill & Mohr, 1962).

Hamster: Spray inhalation of 1-2 mg NDEA twice weekly for 5 months
produced tumours of the trachea and/or lungs in 18/33 Syrian golden
hamsters (Dontenwill et al., 1962). The weekly intratracheal instillation
of 0.05 ml of an aqueous solution (1:14) for a period of up to 6 months
induced tumours in the trachea in 14/14 and in the bronchi in 10/14 animals,
but no liver tumours (Herrold & Dunham, 1963).

(d)   Subcutaneous and/or intramuscular administration

Mouse: S.c. doses of 50 mg/kg bw once or twice weekly, up to a total
dose of 200 or 400 mg/kg bw, significantly increased the frequency of lung
adenomas from 15% in untreated controls to 25-90% in treated animals
(Hilfrich et al., 1971).

Groups of 20 male and 20 female 8-week-old Swiss mice were treated
with single s.c. injections of 0, 2, 4, 8, 16 or 32 mg/kg bw NDEA. In
treated animals, the incidences of lung tumours (including adenomas and
carcinomas) were 16/39, 18/38, 24/39, 25/39 and 21/40, compared with
33/218 in controls; 3 treated mice developed s.c. sarcomas (average
survival, 88 weeks) (Cardesa et al., 1974).

Newborn mouse: Treatment of newborn mice with a single s.c. dose of
50 mg/kg bw caused a significant increase in the number of lung adenomas.
Most of the animals also developed hepatomas within 6 months (Gargus
et al., 1969).

Hamster: Several experiments with Syrian golden hamsters have shown
that NDEA produces carcinomas and papillomas of the upper and lower
respiratory tract (nasal cavity, bronchi, trachea, lungs) and, much less
frequently, tumours of the liver (Dontenwill et al., 1962; Herrold, 1964b,c).
A positive dose-response relationship for tumour induction in the upper
respiratory tract, but not for that in the lower respiratory tract (where
tumour frequency was low), was observed after 12 weekly s.c. doses of

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0.5, 1, 2 or 4 mg/animal; tumour yields in the nasal cavity and the larynx ranged from 17-72% and in the trachea from 88-100%; one liver tumour was observed (Montesano & Saffiotti, 1968). Six different single doses, ranging from 0.75-4 mg/animal, produced papillomas in the trachea, while lower doses, down to 0.03 mg/animal, were without effect when the animals were killed at 25 weeks (Mohr *et al.*, 1966a). A smaller dose (4.6-9.3 mg/kg bw) was found to give a 10% yield of tracheal papillomas in hamsters observed for their lifespan (Dontenwill, 1968).

Twice-weekly s.c. injections of 20 mg/kg bw NDEA to 40 Syrian golden hamsters resulted in 18 neoplasms of the nasal cavity (squamous-cell carcinomas, adenocarcinomas, 'neurogenic tumours'), 31 papillomas of the trachea and 19 tumours of the liver (hepatomas, hepatocellular carcinomas, cholangiocarcinomas); the medium induction time was 190±21.4 days (Mennel *et al.*, 1974).

Daily s.c. injections of 5-20 mg/kg bw NDEA to 3 groups of 5 nursing Syrian golden hamsters for 30 days, commencing on the first day after delivery, caused papillomas in the respiratory tracts (trachea, larynx, bronchi, nasal cavity, lung) of mothers and their offspring. The tumour frequency in the F_1 generation was dependent upon the dose the mother received; the findings indicated that the carcinogen or its metabolites were transmitted to the offspring during lactation (Mohr *et al.*, 1972a).

Tracheal tissue from Syrian golden hamsters pretreated twice weekly with 18 mg/kg bw NDEA for 20 weeks was implanted into their mothers' spleens, and the mothers were subsequently given 18 mg/kg bw NDEA subcutaneously twice weekly for 20 weeks. Tracheal tumours developed within the spleen in the mothers (16/22); three of the tumours were squamous-cell carcinomas (Mohr *et al.*, 1976).

The consistent affinity of NDEA for the respiratory tract was not observed in experiments with Chinese hamsters. A s.c. dose of 77 mg/kg bw, given once weekly for up to 22 weeks to 132 animals, resulted in 82% multiple papillomas of the forestomach and 30% papillomas of the oesophagus; squamous metaplasia but no tumours was observed in the respiratory tract (Mohr *et al.*, 1967).

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In three groups of 40 Chinese hamsters, s.c. treatment with 1/5, 1/10 or 1/20 LD_{50} NDEA (LD_{50} : 230 mg/kg bw) once a week for life produced squamous-cell papillomas of the cheek pouch, tongue, pharynx, oesophagus and forestomach in up to 100% of animals. Carcinomas were also seen occasionally at these sites (Reznik *et al.*, 1976).

Once-weekly s.c. injection of 20 mg/kg bw NDEA for life to 10 wild male European hamsters yielded tumours of the respiratory system in all animals; the main target organ was the nasal cavities. Squamous-cell papillomas were seen in the larynx, trachea and stem bronchi (Mohr *et al.*, 1972b).

Newborn hamster: A single s.c. dose of 0.015, 0.03, 0.09 or 0.15 mg/animal given to newborn hamsters produced tumours of the upper respiratory tract in 30-65% of the animals, but very few tumours were observed in the lungs and bronchi. One liver-cell carcinoma and 1 hepatoma were seen in 144 animals (Montesano & Saffiotti, 1970).

Gerbil: When groups of 40 or 120 gerbils (*Meriones unguiculatus*) were given once-weekly s.c. injections of 6, 12 or 24 mg/kg bw NDEA for life, high incidences (66-80%) of multifocal tumours of the nasal cavity were observed. In addition, papillomas of the tracheobronchial system, adenomas and carcinomas of the lung, as well as cholangiocellular and hepatocellular carcinomas were seen (Cardesa *et al.*, 1976; Haas *et al.*, 1975).

Guinea-pig: S.c. administration of total doses of between 341 and 1310 mg/kg bw produced malignant liver-cell tumours and some benign or malignant tumours in the trachea and ethmoidal region of guinea-pigs (Lombard, 1965).

Hedgehog: S.c. administration of NDEA to Algerian hedgehogs (*Erinaceus*) (total dose, 375-1050 mg/kg bw) resulted in necrosis of the liver parenchyma and benign and malignant tumours of the liver and lung (Graw *et al.*, 1974).

Bird: Grass parakeets were injected intramuscularly once weekly with 100 mg/kg bw for 19 weeks and then once every second week, to a total dose of 2500±600 mg/kg bw; 6/9 birds that survived the treatment died with malignant hepatic tumours (Schmähl *et al.*, 1966).

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(e)   Intraperitoneal administration

Mouse: Two i.p. injections of 100 mg/kg bw produced lung adenomas in 28/29 SWR mice (Mirvish & Kaufman, 1970).

A strain-specific susceptibility to NDEA carcinogenesis was identified by Diwan & Meier (1976a). A single i.p. injection of 90 mg/kg bw led predominantly to lung adenomas (24% *versus* 0% in controls) and leukaemia (68% in both experimental and control animals) in 25 AKR/J mice, to lung adenomas (70% *versus* 22% in controls) and leukaemia (28% *versus* 18% in controls) in 25 SWR/J mice and to 6 hepatomas and 1 bile-duct adenoma in 21 C57BL/6J mice. No liver tumours were seen in 20 C57BL/6J control mice.

Studies on the metastatic tendency of NDEA-induced liver tumours in (C57BL/6JxC3HeB/FeJ)F$_1$ mice revealed metastatic foci in the lungs in 22% (Kyriazis *et al.*, 1974) and 38% of the animals (route unspecified) (Koka & Vesselinovitch, 1974).

Rat: A daily i.p. dose of 0.55 mg/animal for 12 or 23 weeks produced hepatomas in more than 80% of F-344 rats treated (Svoboda & Higginson, 1968).

Hamster: An i.p. dose of 2 mg/animal once a week for 4-7 months produced squamous-cell papillomas of the trachea, epithelial papillomas, carcinomas and neuroepithelial tumours of the nasal cavity, squamous-cell papillomas of the bronchi and hepatic carcinomas (Herrold, 1964b).

Monkey: Two *Ceropethicus aethiops* (green) monkeys treated with 20-40 mg/kg bw every two weeks for 26 months developed hepatic-cell carcinomas (Kelly *et al.*, 1966). It was reported in an abstract that hepatomas and hepatocellular carcinomas were produced by i.p. administration of 40 mg/kg bw once every two weeks for 15 months or longer in all 25 monkeys treated; 17 of these had metastases (O'Gara *et al.*, 1970).

It was reported in an abstract that i.p. administration of NDEA resulted in liver tumours within 8-27 months in rhesus and cynomolgus monkeys. This was preceded by a significant increase in the *alpha*-foeto-protein levels in serum between the third and sixth months (Adamson *et al.*, 1973).

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Mucoepidermoid carcinomas in the nasal cavities occurred in 10/14 prosimian primates (*Galago crassicaudatus*) treated by i.p. injection every 2 weeks with 10-30 mg/kg bw NDEA; 2/10 animals also had primary carcinomas of the liver (Dalgard *et al.*, 1975, 1976).

Newborn monkey: It was reported in an abstract that i.p. injections of NDEA every other week to newborn rhesus monkeys resulted in 100% liver tumour incidence within 10-15 months (Adamson *et al.*, 1974).

(f) Intravenous administration

Rat: A single i.v. injection of 280 mg/kg bw NDEA to 4 rats produced kidney tumours in all animals and one carcinoma of the ovary (Druckrey *et al.*, 1963b, 1964).

The effect upon the kidneys of a single i.v. injection of 1.25-160 mg/kg bw NDEA was studied in groups of 10 male and 10 female Sprague-Dawley rats (Table 2). One female given 1.25 mg/kg bw developed an adenoma of the kidney. Dose levels of 40 mg/kg bw and above increased the tumour incidence and decreased survival time (Mohr & Hilfrich, 1972, 1974).

Gerbil: Single i.v. injections of 50 or 100 mg/kg bw NDEA to groups of 10 male gerbils resulted in the development of carcinomas of the nasal cavities; the tumours originated mainly from the respiratory-olfactory mucosal junction (Cardesa *et al.*, 1976).

(g) Other experimental systems

Prenatal exposure

Pregnant NMRI mice were given NDEA in s.c. doses of 80-240 mg/kg bw from the 15th to the 20th day of gestation; their offspring were killed after 8 or 12 months. A significant increase (up to 63%) in the occurrence of multiple pulmonary adenomas was observed (Mohr & Althoff, 1965a). In addition to lung adenomas, benign and malignant tumours of the liver, oesophagus and forestomach were observed in the offspring of pregnant random-bred or C3H mice treated with NDEA during the last days of pregnancy (Likhachev, 1971, 1974). After treatment with 50 mg/kg bw NDEA on day 18 of gestation, 87% of the offspring of $(AKR/JxSWR/J)F_1$ mice developed pulmonary adenomas (Diwan & Meier, 1976b).

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Table 2^a

Dose mg/kg bw	Females			Males		
	Survival (weeks)	Number of rats with tumours	Number and type of tumours	Survival (weeks)	Number of rats with tumours	Number and type of tumours
1.25	117	1	1 Adenoma	99	-	- -
2.5	108	1	1 Adenoma	98	1	1 Adenoma
5	110	2	2 Adenomas	105	1	1 Adenoma
10	93	-	- -	98	3	3 Adenomas
20	112	1	1 Adenoma	81	-	- -
40	82	$3(1)^b$	3 Adenomas 1 Carcinoma	77	3	2 Adenomas 1 Carcinoma
80	72	6(5)	10 Adenomas 9 Carcinomas 1 Malignant mesen- chymal tumour	87	3(3)	8 Adenomas 1 Carcinoma
160	67	8(7)	13 Adenomas 15 Carcinomas	61	3(2)	4 Adenomas 1 Carcinoma

[a]From Mohr & Hilfrich, 1972
[b]Figures in parentheses are numbers of rats with tumours in both kidneys.

Daily s.c. doses of 4 or 8 mg/animal were given to female Sprague-
Dawley rats from the 10th to the 21st day of pregnancy; 14/26 of the
mothers showed kidney tumours after 1 year, 5 of which were carcinomas.
Some kidney tumours were observed in the offspring at one year of age
(Wrba et al., 1967). Under similar conditions, oral or s.c. administration
to pregnant Wistar rats of varying doses of NDEA produced benign and malig-
nant tumours, mainly thymomas and adenomas of the mammary gland, in the
offspring. Treated female rats died with carcinomas and adenomas of the
kidneys and liver (Thomas & Bollmann, 1968). Daily oral doses of 1 mg/
animal given to female rats before and during pregnancy, up to a total dose
of 60-90 mg/animal, did not result in an increased tumour rate in the off-
spring during lifetime observation; however, 5 carcinomas of the kidney
were observed in 3/4 of the mothers that received a total dose of 60 mg
(Sydow, 1970).

Daily s.c. doses of 2 mg/animal were given to Syrian golden hamsters
for 1-7 days during the second half of the gestation period. Multiple
tracheal papillomas were found in 42% of the young 25 weeks after birth,
and the first tumours appeared when they were between 8 and 12 weeks of
age (Mohr et al., 1965). When 4 mg were given for 2-5 days to mothers,
75% tracheal papillomas were found in the young at 25 weeks (Mohr &
Althoff, 1965b). Of the treated mothers, 73% developed tracheal papillomas
(Mohr et al., 1966b). When Syrian golden hamsters were treated with a
single dose of 45 mg/kg bw NDEA on one of the last four days of pregnancy
(12-15 days), the offspring showed an incidence of up to 95% respiratory
tract tumours, whereas treatment of the mothers during the first 11 days
of pregnancy resulted in no respiratory tract tumours in the offspring
(Mohr et al., 1975).

Oral and subcutaneous administration

The oral administration to a dog of 3 mg/kg bw NDEA, followed by
weekly s.c. injections of the same dose, to a total dose of 565 mg/kg bw,
induced a large leiomyosarcoma of the liver (Schmähl et al., 1964).

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Intrarecetal administration

Twice-weekly treatment of 14 albino rats with 11.2 mg/kg bw for life-time produced hepatocellular carcinomas in all treated animals (Schmähl et al., 1963b).

Intradermal administration

Weekly injections of 3.5 mg NDEA in water for 5-6 months produced epithelial papillomas of the nasal cavity in 10/19 Syrian hamsters; no local tumours were observed (Herrold, 1964a,b).

Immersion

Exposure of the aquarium fish, Brachydanio rerio, to 10-100 mg/l NDEA in the tank-water for 8 weeks resulted in hepatomas or cholangiomas in 17/63 animals (Stanton, 1965). Treatment of guppies (Lebistes reti-culatus) with 13.3-100 mg/l NDEA in the aquarium-water resulted in neo-plastic changes in the liver. After 7-8 weeks, 23% of the fish exhibited liver-cell adenomas, cholangiomas, cholangiocarcinomas or hepatocellular carcinomas (Khudoley, 1971, 1973).

In another study, 60/224 guppies had tumours after application of 26-100 mg/l NDEA in the water for 4-8 weeks. The average latent period was 18 weeks. In the high-dose group (those treated with 100 mg/l for 56 days), mortality was 40-46%; in the low-dosage groups (26-32 mg/l), mortality was 20-28%. Females were more resistant to the toxic effects of NDEA than males (Pliss & Khudoley, 1975).

Levels of 15-135 mg/l NDEA in tank-water for 8 weeks resulted in hepatomas in 21/32 medakas (Oryzias latipes) within the subsequent 5 weeks (Ishikawa et al., 1975).

NDEA in tank-water (50 mg/l) induced hepatocellular carcinomas and adenomas and tumours of the haematopoietic system in 41/94 exposed frogs (Khudoley, 1977).

Carcinogenesis in plants

Seeds of hybrids of *Nicotiana* were soaked in 10 mM solutions of NDEA
in water for 1 or 2 days, and tumours were found on 7.8% of germinated
seedlings 20 days later, compared with 2.1% on controls (Andersen, 1973).

(h) Carcinogenicity of metabolites (see also section 3.2 (a))

N-Nitroso-*N*-ethyl-*N*-(2-hydroxyethyl)amine, which has been detected
in the urine of rats treated with NDEA (Blattmann & Preussmann, 1973),
produced tumours of the liver and a few oesophageal tumours when given
orally to rats (Druckrey *et al.*, 1967).

(i) Factors that modify carcinogenicity in animals

Phenobarbital was reported to decrease the carcinogenic effect of NDEA
and to increase the survival time when the two compounds were administered
concomitantly to NMRI mice. No such effect was observed when halothane or
methoxyflurane were administered together with NDEA, although changes in
the ratio of types of tumours (haemangioendotheliomas and liver-cell
carcinomas) induced by NDEA were observed (Kunz *et al.*, 1969).

A decreased incidence of hepatomas was observed in CF-1 mice given
NDEA neonatally if the neonatal treatment was preceded by prenatal exposure
to methylcholanthrene (Turusov *et al.*, 1973).

In Porton rats, a single i.p. dose of 50-114 mg/kg NDEA 0-2 hours
after partial hepatectomy induced 4/9 liver tumours; 2/9 liver tumours
were induced by 100-130 mg/kg NDEA in intact animals (Craddock 1975). A
high incidence of kidney tumours and a short survival time were observed
in Wistar rats treated with 80 mg/kg bw NDEA 4, 16 or 24 hours after
partial hepatectomy (Meister & Rabes, 1973).

In male Fischer rats, combined treatment with phenobarbital and NDEA
reduced the incidence of hepatocellular carcinomas induced by NDEA, whereas
an opposite effect was observed if phenobarbital was administered one week
after cessation of the hepatocarcinogen. This increased effect was not
modified by concomitant treatment with an antilymphocytic serum (Weisburger
et al., 1975). Treatment of Fischer rats with dibenamine [*N*-(2-chloro-
ethyl)dibenzylamine] during chronic oral treatment with NDEA resulted in a

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decreased number of hepatocellular carcinomas but not of oral, pharnygeal
or oesophageal tumours seen at 25 weeks, when the animals were killed
(Weisburger *et al.*, 1974).

Simultaneous administration to Sprague-Dawley rats of disulfiram (500
mg/kg bw/week) and NDEA (20 mg/kg bw/week) resulted in a significant decrease
in the number of liver tumours and in an increased number of tumours of the
oesophagus and nasal cavities, as compared with rats treated with NDEA alone
(Schmähl *et al.*, 1976). Combined treatment of rats with NDEA and ethanol
resulted in an increased number of tumours of the oesophagus and forestomach
but no change in the induction of hepatomas (Gibel, 1967).

Cyclopropenoid fatty acids have no effect on the carcinogenic response
of rats treated with NDEA (Nixon *et al.*, 1974). A large reduction in the
incidence of liver tumours induced by NDEA was observed in rats when
Kanechlor 500 was administered together with the nitrosamine (Makiura
et al., 1974); no such reduction was observed with 3-methylcholanthrene
or 1-naphthyl isothiocyanate (Makiura *et al.*, 1973).

The incidence of hepatocellular carcinomas induced in rats by NDEA was
reduced by supplementing a marginally deficient diet with lipotropes or
amino acids (both containing methionine) (Rogers, 1977), but was enhanced
by a diet high in fat and marginally deficient in lipotropes (Rogers *et al.*,
1974).

In Syrian golden hamsters given benzo[a]pyrene and ferric oxide intra-
tracheally, followed by s.c. administration of NDEA, a higher incidence of
squamous-cell carcinomas of the tracheobronchial tract was found as compared
with hamsters treated with the carcinogens singly (Montesano *et al.*, 1974).

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

The acute oral or i.v. LD_{50} of NDEA in rats was 280 mg/kg bw (Druckrey
et al., 1967). The s.c. LD_{50} was 250 mg/kg bw in European hamsters (Mohr
et al., 1972b) and 230 mg/kg bw in Chinese hamsters (Reznik *et al.*, 1976).

Toxic doses of NDEA produce acute haemorrhagic centrilobular necrosis
of the liver. The toxicity and the inhibitory effects on protein and
nucleic acid synthesis in the liver and other organs have been reviewed
(Magee & Barnes, 1967; Magee & Swann, 1969; Magee *et al.*, 1976;
Witschi, 1973).

Feeding of NDEA to rats resulted in the development of histologically
distinct foci in the liver, referred to as 'enzyme-deficient islands'
(Friedrich-Freksa *et al.*, 1969a,b; Schauer & Kunze, 1968). These islands
are considered to be precursors of liver-cell carcinomas. NDEA induces a
a hepatocellular population which is resistant to the necrogenic effect
of a second carcinogen, 2-acetylaminofluorene (Solt & Farber, 1976).

Embryotoxicity and teratogenicity

Teratogenic effects were not observed in rats given single doses of
180 mg/kg bw intraperitoneally or 200 mg/kg bw orally during pregnancy.
An increase in foetal mortality was noted when the substance was admini-
stered on days 3, 9, 10 or 12 of gestation. Similar foetal mortality was
observed after an intraplacental injection of 0.1-0.3 mg NDEA to every
embryonic chamber on day 13 of gestation (Alexandrov, 1974; Napalkov &
Alexandrov, 1968).

Absorption, distribution and excretion

In goats, one hour after oral administration of 30 mg/kg bw NDEA,
there were 11.4 mg/kg NDEA in the milk and 11.9 mg/kg in the blood.
Only traces were found in the milk and none in the blood after 24 hours
(Juszkiewicz & Kowalski, 1974).

Metabolism

Available evidence suggests that NDEA requires metabolic activation
in order to exert its carcinogenic and toxic effects. Such activation has
been demonstrated both *in vitro* and *in vivo*. Tissue distribution and
blood concentration have been followed in rats (Magee & Barnes, 1967;
Rajewsky & Dauber, 1970); and metabolism has been monitored by measurement
of the rate of loss from the blood and by $^{14}CO_2$ exhalation after admini-

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stration of ^{14}C-NDEA (Heath, 1962; Mundt & Hadjiolov, 1974). A dose of 200 mg/kg bw was metabolized in rats during 24 hours (Heath, 1962).

N-Nitrosoethyl-N-(2-hydroxyethyl)amine and N-nitrosoethyl-N-(carboxy-methyl)amine have been detected in the urine of rats given NDEA (Blattmann & Preussmann, 1973).

Oxidative N-deethylation of NDEA accounts for the production of CO_2 and alkylating species in $vivo$ (Heath, 1962; Swann & Magee, 1971). The rate of metabolism of NDEA by slices of organs from rats and hamsters in $vitro$ has been measured, and a correlation made between the degree of metabolism and the distribution of induced tumours (Montesano & Magee, 1974).

After administration of NDEA to rats or hamsters, several ethylated derivatives were produced in liver and kidney nucleic acids. These inclu-ded 7-ethylguanine, 0^6-ethylguanine and 3-ethyladenine (Magee & Lee, 1964; Montesano & Bartsch, 1976; Pegg, 1977; Swann & Magee, 1971).

Treatment with aminoacetonitrile reduced the ethylation of liver RNA and the exhalation of $^{14}CO_2$ after a single i.p. injection of ^{14}C-NDEA (Mundt & Hadjiolov, 1974).

NDEA incubated in the presence of a rat liver microsomal system and 3,4-dichlorothiophenol as a trapping agent yields the corresponding methyl and ethyl thioethers (Preussmann et $al.$, 1976).

Mutagenicity and other short-term tests

The genetic effects of NDEA have been reviewed (Montesano & Bartsch, 1976).

NDEA, in the presence of microsomal fractions from mouse liver or with Udenfriend's oxidation system, caused forward mutations in $Saccharomyces$ $cerevisiae$ and $Neurospora$ $crassa$ (Malling, 1966; Mayer, 1971). It produced reverse mutations in $Salmonella$ $typhimurium$ strains TA 100 and TA 1530 (Bartsch et $al.$, 1975; Sugimura et $al.$, 1976) and in $Escherichia$ $coli$ in the presence of a rat liver microsomal fraction from phenobarbital- or polychlorinated biphenyl-treated rats (Nakajima et $al.$, 1974).

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N-Nitrosoethyl-N-(α-acetoxyethyl)amine, which is readily converted into the unstable α-hydroxyethyl derivative, is mutagenic in *Drosophila melanogaster* (Fahmy & Fahmy, 1976) and in *S. typhimurium* TA 1530 in the absence of a liver activation system (Camus *et al.*, 1978).

In host-mediated assays in mice given doses of 50 mg/kg, tests on *S. typhimurium* demonstrated mutagenic effects (Malling, 1974).

In the presence of a liver microsomal fraction from phenobarbital-treated rats, NDEA caused 8-azaguanine-resistant mutants in Chinese hamster V79 cells (Kuroki *et al.*, 1977).

NDEA was mutagenic in the recessive lethal test in *Drosophila melanogaster* (Pasternak, 1963). The induction of point mutations was a function of concentrations over more than two orders of magnitude. The ability of NDEA to produce more than one point mutation per germ cell contrasts with the lack of dominant lethals, translocations and chromosome loss seen with concentrations below toxic levels (LD_{60}-LD_{90}) (Fahmy *et al.*, 1966; Vogel & Leigh, 1975).

Dominant lethal mutations were not observed in mice treated with 13.5 mg/kg bw NDEA (Propping *et al.*, 1972).

In the presence of a rat liver microsomal system *in vitro*, 4-200 mM NDEA induced chromosomal aberrations as well as sister chromatid exchanges in Chinese hamster cells (Natarajan *et al.*, 1976).

NDEA did not induce transformation of hamster embryo cells; however, treatment of the embryo by transplacental administration of NDEA led to the appearance of transformed cells in the primary culture. These transformed cells were produced at a much higher frequency than in control cultures from untreated embryos, and they produced tumours (fibrosarcomas) when injected back into animals (DiPaolo *et al.*, 1972; Evans & DiPaolo, 1975).

(b) Humans

No data were available to the Working Group.

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3.3 Case reports and epidemiological studies

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

N-Nitrosodiethylamine is carcinogenic in all animal species tested:
mice, rats, Syrian golden, Chinese and European hamsters, guinea-pigs,
rabbits, dogs, gerbils, pigs, monkeys, hedgehogs, various fish, frogs and
birds. It induces benign and malignant tumours after its administration
by various routes, including ingestion, parenteral injection, inhalation
and rectal instillation. The major target organs are the liver, respira-
tory and upper digestive tracts and kidney. It is carcinogenic following
its administration prenatally and in single doses. In several studies,
dose-response relationships were established.

N-Nitroso-*N*-ethyl-*N*-(2-hydroxyethyl)amine, a metabolite of *N*-nitroso-
diethylamine, produced mainly liver tumours after its oral administration
to rats.

4.2 Human data

No case reports or epidemiological studies were available to the
Working Group. Available information on occurrence suggests that the
general population may be exposed to low levels of *N*-nitrosodiethylamine;
however, no exposed group suitable for an epidemiological investigation
has yet been identified.

4.3 Evaluation

There is *sufficient evidence* of a carcinogenic effect of *N*-nitrosodi-
ethylamine in many experimental animal species. Although no epidemiological
data were available, *N*-nitrosodiethylamine should be regarded for practical
purposes as if it were carcinogenic to humans.

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N-NITROSODIMETHYLAMINE

This substance was considered previously by an IARC Working Group, in December 1971 (IARC, 1972). Since that time new data have become available, and these have been incorporated into the monograph and taken into account in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 62-75-9

Chem. Abstr. Name: *N*-Methyl-*N*-nitrosomethanamine

N,N-Dimethylnitrosamine; dimethylnitrosamine; DMN; DMNA; NDMA

1.2 Structural and molecular formulae and weight

$$O=N-N\begin{cases} CH_3 \\ CH_3 \end{cases}$$
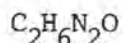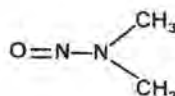
$C_2H_6N_2O$ Mol. wt: 74.1

1.3 Chemical and physical properties of the pure substance

(a) Description: Yellow, oily liquid (Magee & Barnes, 1967)

(b) Boiling-point: 50-52°C (14 mm); 151°C (760 mm) (Druckrey *et al.*, 1967)

(c) Density: d_4^{20} 1.0061

(d) Refractive index: n_D^{20} 1.4368

(e) Spectroscopy data: λ_{max} 230 and 332 nm (E_1^1 = 978.9 and 12.8) in water (Druckrey *et al.*, 1967); mass spectroscopy data are given by Pensabene *et al.* (1972) and Rainey *et al.* (1978).

(f) Solubility: Soluble in water, organic solvents and lipids

(g) Volatility: Volatile; can be steam-distilled quantitatively (Eisenbrand *et al.*, 1970)

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(h)   Stability: Stable at room temperature for more than 14 days
in neutral or alkaline aqueous solutions in the dark (Druckrey
et al., 1967);   slightly less stable in acidic solutions;
sensitive to ultra-violet light

(i)   Reactivity: Strong oxidants (peracids) oxidize it to the
corresponding nitramine;   can be reduced to the corresponding
hydrazine and/or amine;   relatively resistant to hydrolysis
but can be easily split by hydrogen bromide in acetic acid
(Eisenbrand & Preussmann, 1970).   Photochemically reactive
(Fridman *et al.*, 1971).   The preparation of various derivatives
has been discussed (Preussmann *et al.*, 1978).

1.4   Technical products and impurities

No data were available to the Working Group.

2.   Production, Use, Occurrence and Analysis

2.1   Production and use

For background information on this section, see preamble, p. 22.

(a)   Production

N-Nitrosodimethylamine (NDMA) was first prepared in 1895 by Renouf
by the reaction of sodium nitrite with an acidified solution of dimethyl-
amine hydrochloride (Prager *et al.*, 1922).   Essentially the same procedure
is believed to have been used for its commercial production (Getz, 1960).

Production of NDMA has not been reported to the US International
Trade Commission;   however, prior to 1 April 1976, one US company produced
it as an intermediate in the manufacture of 1,1-dimethylhydrazine[1] (unsym-
metrical dimethylhydrazine) (Anon., 1975).   Prior to closing of this plant,
annual production of NDMA is believed to have been less than 500 thousand
kg per year.   Production of 1,1-dimethylhydrazine was first reported to
the US International Trade Commission in 1956 (IARC, 1974).   1,1-Dimethyl-

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[1]See IARC (1974).

hydrazine is also believed to have been produced in Germany in the early 1940's, but no information is available about the route of synthesis. No evidence was found that NDMA has been produced commercially in Japan, and it is not known if it is produced in other countries.

(b) Use

Prior to 1 April 1976, NDMA was used in the US as an intermediate in the production of 1,1-dimethylhydrazine, a storable liquid rocket fuel which is believed to have contained up to 0.1% NDMA as an impurity. No evidence was found that NDMA is used at present, except for research purposes.

Regulations in the US concerning NDMA designate strict procedures to avoid worker contact. Mixtures containing 1.0% or more NDMA must be maintained in isolated or closed systems, employees must observe special personal hygiene rules, and certain procedures must be followed for movement of the material and in case of accidental spills and emergencies (US Occupational Safety and Health Administration, 1976).

2.2 Occurrence

(a) Air

Bretschneider & Matz (1974, 1976) reported levels of 1-430 ng/m^3[1] NDMA inside a factory using dimethylamine. Fine *et al.* (1976a) reported its presence as an air pollutant in Baltimore, Maryland and in Belle, West Virginia (USA). In Baltimore, the prime source was found to be a chemical plant which was manufacturing 1,1-dimethylhydrazine using NDMA as a precursor. Typical NDMA levels were between 6000 and 36,000 ng/m^3 on the site of the factory, about 1000 ng/m^3 in residential neighbourhoods adjacent to the factory, and about 100 ng/m^3 two miles away in downtown Baltimore (Fine, 1978; Fine *et al.*, 1976b,c, 1977a; Pellizzari *et al.*, 1976). Following this study, in April 1976, the factory was closed down. In Belle, the source of the NDMA was found to be a chemical factory manufacturing and

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[1]This result was not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).

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using dimethylamine;   the NDMA was being produced as an unwanted byproduct.
Typical levels in downtown Belle and Charleston ranged from 1-40 ng/m^3
(Fine, 1978).   Similar levels[1] were found by Cohen & Bachman (1978) on the
site of several chemical factories making or using dimethylamine.   In
extensive studies in New York City, Boston and New Jersey, NDMA was found
at 3/40 sites at levels above the detection limit of 10 ng/m^3 (Fine, 1977,
1978).   Gough *et al.* (1976) and Sen *et al.* (1976a) have found that during
the frying of bacon, 70% of NDMA volatilized in the fumes.

(b)   Tobacco smoke

Johnson & Rhoades (1972) and Rhoades & Johnson (1972) claimed to
have found 0-140 ng/cigarette NDMA [One of these results was confirmed
by high-resolution mass spectrometry].   Similar results were reported by
McCormick *et al.* (1973), using high-resolution mass spectrometry.   Klimsch
et al. (1976) reported 9.5-91 ng/cigarette.

NDMA was present in the mainstream smoke of non-filtered cigarettes
at a level of 13-65 ng/cigarette, and 5.7-43 ng/cigarette were found in
filtered cigarettes.   In the sidestream smoke, the levels were 680-823 ng/
cigarette in non-filtered cigarettes and 1040-1770 ng/cigarette in filtered
cigarettes.   In smoke-filled rooms, such as bars and discotheques, 90-240
ng/m^3 NDMA[1] were found in the air.   In residences, the levels[1] were <5 ng/m^3
(Brunnemann & Hoffmann, 1978;   Brunnemann *et al.*, 1977).   Walker & Castegnaro
(IARC, 1977c) found NDMA in all of 10 samples of scrapings from pipes used
for smoking tobacco in the Transkei;   these are believed to be consumed by
the local inhabitants.   The levels ranged from 45-340 µg/kg.

(c)   Water

NMDA was present in sea-water adjacent to a 1,1-dimethylhydrazine
chemical factory in Baltimore which was emitting NDMA into the air;   the
levels varied from 0.08-0.25 µg/l (Fine & Rounbehler, 1976).   NDMA was also
reported at a level of 3.0 µg/l in an adjacent sewage-treatment facility.

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[1]These results were not confirmed by mass spectroscopy (see also
'General Remarks on the Substances Considered', p. 40).

On the site of the Baltimore factory, water in a drainage ditch contained 6 µg/l and that in a mud puddle 200 and 6000 µg/l (Fine *et al.*, 1977a). Industrial waste-water from chemical factories was found to contain 0.2-5 µg/l (Fine, 1978); that from 8/19 chemical factories contained 0.08-3.3 µg/l NDMA (Cohen & Bachman, 1978).

Cohen & Bachman (1978)[1], Gough (1978)[1] and Fiddler *et al.* (1977) found 0.012-0.34 µg/l in deionized water; the source of the NDMA was shown to be the ion-exchange resin. Fine (1978) reported <0.01 µg/l[1] NDMA in high-nitrate well-water. Cohen & Bachman (1978) also reported that chlorination of drinking-water can result in NDMA levels of 0.02-0.82 µg/l[1].

(d) Soil

Soil samples taken from several locations near industrial plants in New Jersey contained NDMA at levels ranging from 0-15.1 ng/g; in West Virginia, near Belle and Charleston, soil samples contained 0.2-5.4 ng/g; and in the New York City area, soil samples contained 0-0.32 ng/g (Fine, 1977).

(e) Food and feed

Cheese: Various cheeses have been analysed for NDMA. Havery *et al.* (1976) found none, and Alliston *et al.* (1972) found only a trace. However, Sen *et al.* (1978) found 2-68 µg/kg NDMA in 20/63 samples of 26 different cheeses. Eisenbrand *et al.* (1978) found that 78/173 cheeses were positive for NDMA, and levels of 1-6 µg/kg were confirmed in 20 of these samples. Gough (1978) found up to 0.2 µg/kg in 13/20 samples, and 3/16 samples of other dairy products contained NDMA at levels of <0.1 µg/kg.

Vegetables and vegetable oils: Telling *et al.* (1974) found no NDMA in raw or cooked vegetables, and Gough (1978) found no NDMA in 16 different vegetable samples. None was found in baby food or lard (Havery *et al.*, 1976), but freshly refined soya bean oil was found to contain levels of 0-20 µg/kg[1] (Hedler *et al.*, 1976).

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[1]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).

NDMA was found in 5/12 samples of tinned fruit at a level of <0.1 µg/kg (Gough, 1978) and in the fruit of a solanaceous bush, *Solanum incanum*, used in the Transkei for the preparation of foodstuffs (Du Plessis *et al.*, 1969)[1].

In a dietary survey to compare levels of nitrosamines in regions of high and low incidence of oesophageal cancer, no significant difference in NDMA levels was found among 179 samples. Levels were mostly <5 µg/kg[1] (IARC, 1975; Joint Iran-IARC Study Group, 1977).

Meat and fish: The presence of NDMA in meat and fish products has been demonstrated in a number of studies (Table 1).

Spices: Sen *et al.* (1974) found 0-850 µg/kg NDMA in spices used for meat curing, and Havery *et al.* (1976) reported 29-343 µg/kg in spice cures. No nitrosamines were found in the mixtures when the spices and nitrite and/or nitrate salts were packaged separately.

Feed: Among the first reports of nitrosamines in the environment were those of Sakshaug *et al.* (1965) and Ender *et al.* (1964), who found high levels of NDMA in fishmeal, using thin-layer chromatography. Mirna *et al.* (1976) reported 2, 3, 8, 18, 205 and 315 µg/kg NDMA. Fishmeal not treated with nitrate or nitrite was found to contain between 150 and 1000 µg/kg NDMA (Hurst, 1976). Skaare & Dahle (1975) reported 100-2000 µg/kg NDMA, and Juszkiewicz & Kowalski (1976) 5-417 µg/kg[1].

Levels of 0-42 µg/kg NDMA have been found in a number of experimental animal feeds which contain fishmeal (IARC, 1977a). Kann *et al.* (1978) reported NDMA in 37/46 animal feeds: 23 samples contained 1-10 µg/kg and 14, more than 10 µg/kg (one of these had 79 µg/kg).

(f) Alcoholic beverages

Examination of over 100 apple brandies has shown that NDMA was present in a majority of samples. The average level found was 0.5 µg/kg with a maximum of 10 µg/kg. NDMA was also found in a number of ciders, cognacs,

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[1]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).

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Table 1

Levels of N-nitrosodimethylamine in various foods from several countries[1]

Product	Country	No. of samples	Number positive	Level (µg/kg)	Reference
Various meat products	Canada	59	5	10–80	Sen (1972)
" " "	"	197	57	2–12	Panalaks et al. (1973)
" " "	"	80	29	2–35	" " (1974)
" " "	"	100	26	5–48	Sen et al. (1976b)
Bacon (fried)	"	8	7	2–5	" " (1973)
Fish (fresh)	"	18	11	3–18	Iyengar et al. (1976)
" (processed)	"	11	3	3–6	" " "
Cured meats (raw)	FRG	34	26	<1–2.4	Eisenbrand et al. (1978)
" " (cooked)	"	34	20	<1–1.7	" " "
Bacon (raw)	"	5	5	1–3	" " (1977)
" (cooked)	"	5	5	1–4	" " "
Liver, meatloaf (raw)	"	4	4	1–8	" " "
" " (cooked)	"	4	2	1	" " "
Pepper, salami (raw)	"	5	3	1–10	" " "
" " (cooked)	"	5	5	1–12	" " "
Ham (raw)	"	6	5	1–54	" " "
" (cooked)	"	4	4	1–6	" " "
Salted fish	Hong Kong	21	8	1–35	Huang et al. (1978a,b)
Fish products	" "	61	27	1–15	Fong & Chan (1977)
Croakers (fish)	" "	2	1	20–30[2]	" " (1973)

[1]No data were available to the Working Group from other countries.
[2]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).

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Product[*]	Country	No. of samples	Number positive	Level (μg/kg)	Reference
Cured meats	The Netherlands	5	5	0.0-27.3	Stephany *et al.* (1976)
" "	"	30	7	2-6	Groenen *et al.* (1976)
Bacon (fried)	"	5	2	2-4	" " "
" "	"	5	5	0.3-1.1	Stephany *et al.* (1976)
Meat (fried)	"	5	5	0.0-0.4	" " "
Bacon (raw)	"	8	7	0.9-9.5	Groenen *et al.* (1977)
" (fried)	"	7	7	1.2-7.5	" " "
Minced meat (raw)	"	5	0	-	" " "
" " (fried)	"	5	5	0.4-1.9	" " "
Cured meats	"	22	22	0.1-15.5	" " "
Stale cod	UK	2	2	1[2]	Alliston *et al.* (1972)
Bacon (grilled/fried)	"	29	NR[3]	>1-3	Telling *et al.* (1974)
" (cooked)	"	16	NR	<0.1-6	Gough & Walters (1976)
" (cooked-out fat)	"	42	NR	<0.2-10	Telling *et al.* (1974)
" " " "	"	16	NR	<0.1-28	Gough & Walters (1976)
Spiced tinned meat	"	NR	5	3-5	Gough (1978)
Various meat products	"	35	NR	<0.2-<1	Telling *et al.* (1974)
" " "	"	6	NR	<0.5[2]	Alliston *et al.* (1972)
Sausages (smoked)	"	4	NR	<0.2-<1	Telling *et al.* (1974)
Ham (tinned)	"	2	NR	<0.2	" " "
Bacon (raw)	"	2	NR	<0.5[2]	Alliston *et al.* (1972)
" "	"	10	NR	<0.2	Telling *et al.* (1974)
" (grilled/fried)	"	24	5	1-4	Crosby *et al.* (1972)
" (fried)	"	8	8	1-10	Gough (1978)
" (grilled/fried)	"	46	13	1-5	Gough *et al.* (1977)

[2]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).
[3]NR = not reported

Product	Country	No. of samples	Number positive	Level (μg/kg)	Reference
Cured meat (tinned)	UK	34	9	1-10	Gough *et al.* (1977)
Salami, sausage	"	22	2	1-5	" " "
Poultry (cooked)	"	9	1	1-5	" " "
Fish (uncooked)	"	61	23	1-10	" " "
" (cooked)	"	9	0	<1[4]	" " "
Cheese	"	58	10	1-15	" " "
Yoghurt and dessert dishes	"	16	0	<1[4]	" " "
Fish (fresh)	"	85	NR[3]	<0.2-9	Telling *et al.* (1974)
" (tinned)	"	16	NR	<0.2-2.5	" " "
" and fish products	"	35	15	1-9	Crosby *et al.* (1972)
" " " " "	"	20	10	up to 0.2[2]	Gough (1978)
Japanese salmon (raw)	USA	24	0	<5[4]	Gadbois *et al.* (1976)
Fin fish	"	26	0	<10[4]	Havery & Fazio (1977)
Shellfish	"	52	0	<10[4]	" " "
Fish and fish products	"	26	22	4-26	Fazio *et al.* (1971a)
Various meat products	"	51	1	5	" " (1971b)
Frankfurters	"	40	3	0-84	Wasserman *et al.* (1972)
Souse, jellied cured meats	"	10	8	3-63	Fiddler *et al.* (1975)
Various cured meats	"	39	0	<10[4]	Havery *et al.* (1976)
Bacon (raw and fried)	"	22	0	<10[4]	" " "
Various cured meats	"	106	some	<1	" " (1978)
Sausages	USSR	12	9	1-13[2]	Kann *et al.* (1976)
Fish (fresh and processed)	"	19	18	6-177[2]	" " "

[2]These results were not confirmed by mass spectroscopy (see also 'General Remarks on the Substances Considered', p. 40).
[3]NR = not reported
[4]Detection limited

armagnacs, rums and whiskeys;   average levels ranged from 0.1-0.4 µg/kg
with a maximum of 1.6 µg/kg.

In 27 beers examined, the average level was 2 µg/kg, with a maximum
of 7 µg/kg (IARC, 1977b).   NDMA was detected in 3/18 samples of maize beer
at levels of 1.0, 1.5 and 7.5 µg/kg (IARC, 1976).

(g)   Pesticides

Fine et al. (1978) reported 190-640 mg/1 (ppm) NDMA in dimethylamine
formulations of 2,3,6-trichlorobenzoic acid;   300 µg/1 NDMA were also found
in 1/3 herbicide mixtures formulated as dimethylamine salts.   Cohen et al.
(1978) reported levels ranging from 0.1-335 mg/kg (ppm) NDMA in 7/8 di-
methylamine salts of various herbicides.

(h)   Drugs

Eisenbrand et al. (1978) found NDMA in all samples of 68 drugs
formulated with aminopyrine, including tablets, suppositories, injections,
drops and syrups.   The concentration varied within wide limits (<10-371
µg/kg):   35 samples contained 1-10 µg/kg, 27 samples contained 10-50 µg/kg,
5 samples contained 50-100 µg/kg and 1 sample (a tablet) contained 371 µg/kg.

(i)   In vivo formation

NDMA was formed in laboratory mice in vivo following gavage with
250 ng sodium nitrite then 50 ng dimethylamine hydrochloride (Rounbehler
et al., 1977).

NDMA has been reported to be present in man in vivo.   Harington et al.
(1973) claimed that NDMA was present in vaginal fluids [Confirmation was
by low-resolution mass spectrometry, and the results have not been reported
elsewhere.   No adequate precautions were taken to prevent nitrosamine
formation during analysis].

Eisenbrand et al. (1976) reported the presence of 10 and 100 µg/1
NDMA in 2/8 urine samples from patients with urinary tract infections;
Hicks et al. (1978) reported traces in the urine of some patients with
chronic bladder infections (detection limit, 1 µg/kg);   Stephany & Schuller
(1978) reported levels of <1 µg/kg in the urine of hospital patients treated

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with potassium nitrate; and similar results[1] have been reported by Brooks
et al. (1972) and Radomski & Hearn (1976) [In none of this work were
adequate precautions taken to prevent nitrosamine formation during
storage and analysis].

Lakritz *et al.* (1978) reported the presence of 2 µg/kg NDMA in the
gastric juice from 2/35 fasting patients [No adequate precautions were
taken to prevent nitrosamine formation during storage and analysis].

Fine *et al.* (1977c) found more NDMA in human blood immediately after
ingestion of a meal containing spinach, bacon, tomato, bread and beer
than was present before. Ingestion of ascorbate reduced the background
amount to a non-detectable level.

2.3 Analysis

An IARC Manual gives selected methods for the analysis of volatile
N-nitrosamines, including NDMA (Preussmann *et al.*, 1978). Four techniques
have been used for trapping NDMA during air monitoring: charcoal traps
(Bretschneider & Matz, 1974), Tenax GC cartridges (Issenberg & Sornson,
1976; Pellizzari *et al.*, 1976), cryogenic traps (Fine *et al.*, 1976c)
and ambient temperature alkali traps (Fine *et al.*, 1977a).

On 29 September 1977, the US Environmental Protection Agency issued a
notice requiring all registrants of pesticide products that are potentially
contaminated with *N*-nitroso compounds to analyse commercial samples which
have been stored for at least 18 days to determine the extent of contamin-
ation. This notice prescribes in general terms the types of analytical
methods to be used for volatile *N*-nitroso compounds (e.g., gas chromatography
plus mass spectrometry or thermal energy analysis and others) as well as for
nonvolatile *N*-nitroso compounds (e.g., high-pressure liquid chromatography
plus ultra-violet spectroscopy). Confirmation of positive results by gas
chromatography and mass spectrometry or by valid independent methods is
required when possible (US Environmental Protection Agency, 1977).

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[1]These results were not confirmed by mass spectroscopy (see also
'General Remarks on the Substances Considered', p. 40).

3.   Biological Data Relevant to the Evaluation
of Carcinogenic Risk to Man

3.1 Carcinogenicity and related studies in animals

(a) Oral administration

Mouse: Several studies on different strains of mice have demonstrated
that N-nitrosodimethylamine (NDMA) produces haemangiomas, haemangioendo-
theliomas, haemangioendothelial sarcomas, adenomas and hepatocellular
carcinomas of the liver, as well as adenomas and adenocarcinomas of the
lung. Kidney adenomas have also been observed in some strains (Clapp &
Toya, 1970; Clapp et al., 1968, 1971; Den Engelse et al., 1969/1970;
Kuwahara et al., 1972; Otsuka & Kuwahara, 1971; Shabad & Savluchinskaya,
1971; Takayama & Oota, 1963, 1965; Terracini et al., 1966; Toth et al.,
1964; Zwicker et al., 1972). A concentration of 50 mg/l NDMA in the
drinking-water for one week was sufficient to induce tumours in the kidney
and lung (Terracini et al., 1966). The lowest dose tested in long-term
studies, a concentration in the drinking-water that corresponded to a
dose of 0.4 mg/kg bw/day (total dose, 89 mg/kg bw), produced 13/17 lung
adenomas and 2/10 haemangiocellular tumours in male RF mice (Clapp & Toya,
1970).

Rat: The carcinogenicity of NDMA has been demonstrated in several
different strains of rat. Although differences have been seen in target
organs, a consistent observation has been that long-term treatment with
doses compatible with good survival rates, i.e., not more than 50-100 mg/kg
in the diet or drinking-water, or 4 mg/kg bw/day, leads to the development
of high incidences of hepatocellular carcinomas and cholangiocellular
tumours (Argus & Hoch-Ligeti, 1961; Geil et al., 1968; Magee & Barnes,
1956, 1962; Schmähl & Preussmann, 1959; Terracini et al., 1967). Haeman-
gioendothelial sarcomas of the liver have also been observed (Hadjiolov &
Markow, 1973; Taylor et al., 1974). Short-term or single-dose treatment
with high doses (100-500 mg/kg of diet, or up to 30 mg/kg bw) produces
kidney tumours (Magee & Barnes, 1959, 1962; Riopelle & Jasmin, 1969;
Shinohara et al., 1976; Terracini et al., 1969; Zak et al., 1960);
the histology of these tumours has been described in detail (Ireton et al.,

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1972; Ito, 1973; Ito *et al.*, 1971; Jasmin & Cha, 1969; Jasmin & Riopelle, 1968, 1969; McGiven & Ireton, 1972). Lung adenocarcinomas and squamous-cell carcinomas have been seen occasionally after NDMA treatment (Argus & Hoch-Ligeti, 1961; Zak *et al.*, 1960).

A dose-response study has been carried out in Porton rats in which NDMA in oil solution was added to the diet. After an observation time of up to 120 weeks, the following frequencies of liver tumours (mainly liver-cell carcinomas) were observed (Table 2) (Terracini *et al.*, 1967):

Table 2[a]

Dose-response study with *N*-nitrosodimethylamine in rats

NDMA mg/kg of diet	Number of rats initially	Number of rats with liver tumours
0 (control)	41	0
2	37	1
5	68	5
10	5	2
20	23	15
50	12	10

[a]From Terracini *et al.*, 1967

Treatment of Sprague-Dawley rats with 0.4 mg/rat 5 times weekly for 24 weeks (total dose, 48 mg/rat) induced liver tumours (3 liver-cell carcinomas, 2 sarcomas) in 5/19 animals (Hoch-Ligeti *et al.*, 1968).

Hexadeutero-NDMA, at a level of 5 mg/l in the drinking-water, induced a 3% incidence of liver carcinomas, while NDMA under identical conditions induced 26% (Keefer *et al.*, 1973).

Hamster: Administration by stomach tube of 1.6, 1.0 and 1.0 mg/animal NDMA over 5 weeks, or of 1 dose of 1.6 mg/animal, to Syrian golden hamsters induced cholangioadenomas, cholangiocarcinomas and haemangio-

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sarcomas as well as haemangioendotheliomas of the liver (Tomatis & Cefis, 1967). Liver-cell carcinomas, cholangiocarcinomas and haemangioendotheliomas were obtained by giving 25 mg/l NDMA in the drinking-water for 11 weeks (Kowalewski & Todd, 1971; Tomatis *et al.*, 1964). Administration of 1 mg/l NDMA in the drinking-water to inbred Syrian hamsters (strain B10® 87.29) for 60 weeks induced 1 adenocarcinoma of the glandular stomach in 40 animals (Homburger *et al.*, 1976).

Guinea-pig: Male guinea-pigs given 25 or 50 mg NDMA/kg of diet for 6-49 weeks developed papillary cholangiomas and liver-cell carcinomas (Le Page & Christie, 1969a).

Rabbit: Doses of 25 and 50 mg NDMA/kg of diet given to rabbits for 17-60 weeks resulted in hepatocellular carcinomas with lung metastases and benign papillary cholangiomas (Le Page & Christie, 1969b).

Duck: It was reported in an abstract that treatment with diet containing 50 mg NDMA/kg of diet to male Peking ducks for 8-10 months resulted in an incidence of 71% anaplastic haemangiosarcomas of the liver after 9 months (McCracken *et al.*, 1973).

Fish: Doses of 300, 1200, 4800 and 19,200 mg NDMA/kg of diet given to rainbow trout for more than 6 months induced adenomas and adenocarcinomas of the liver (Ashley & Halver, 1968). Guppies (*Lebistes reticulatus*) received 4.8 g NDMA/kg of diet for several months; after 13 months, 2/20 developed hepatic nodules, described as liver tumours, and a leiomyosarcoma in the mesentery (Sato *et al.*, 1973).

(b) Inhalation and/or intratracheal administration

Mouse: BALB/c mice were exposed daily to 0.005 or 0.2 mg/m^3 NDMA for 17 months. Tumours were observed earlier and in larger amounts in the lung, liver and kidney only in those given the higher concentration (Moiseev & Benemansky, 1975).

Rat: Twice-weekly inhalation of NDMA at a concentration corresponding to 4 mg/kg bw for 30 minutes induced tumours (aesthesioneuroepitheliomas and squamous-cell carcinomas) of the ethmoturbinals in 4/6 BD rats. With half the concentration, 8/12 rats developed tumours of the nasal cavity

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(Druckrey *et al.*, 1967). In Wistar rats exposed daily by inhalation to 0.005 or 0.2 mg/m³ NDMA for 25 months, those given the higher level had tumours in the lung, kidney and liver earlier and in larger amounts than in controls (Moiseev & Benemansky, 1975).

(c) Subcutaneous and/or intramuscular administration

Mouse: Weekly injections to DD, BALB/c and SJL/J strain mice of 0.15 mg NDMA in 0.2 ml saline for 1-25 weeks (total dose, 0.15-3.75 mg/ animal) induced haemangioendothelial sarcomas of the liver and of the retroperitoneal and abdominal soft tissues, and adenomas or adenocarcinomas of the lung (Kuwahara *et al.*, 1972; Otsuka & Kuwahara, 1971). After single s.c. administrations of NDMA, a dose-response relationship was seen for lung tumours (adenomas and carcinomas): 1 mg/kg bw = 29%, 2 mg/kg bw = 35%, 4 mg/kg bw = 39% and 8 mg/kg bw = 67% (Cardesa *et al.*, 1974a).

Newborn and suckling mouse: Following single s.c. injections of 15-75 µg/animal, mice developed parenchymal-cell and vascular tumours of the liver as well as lung adenomas (Terracini *et al.*, 1966; Toth & Shubik, 1967; Toth *et al.*, 1964).

Rat: Injections of 10, 20 or 30 mg/rat at day 21 or 70 after birth produced 38% kidney tumours after median latent periods of 286-369 days; 41% of kidney tumours were of the renal cell type and 59% were stromal nephromas. No control rats developed kidney tumours (Campbell *et al.*, 1974).

Newborn and suckling rat: Single doses of 0.125 mg NDMA/animal induced tumours of the kidney and hepatocellular carcinomas (Terracini & Magee, 1964; Terracini *et al.*, 1969).

Forty weekly s.c. injections to Wistar rats of 0.1 mg/rat, beginning at birth, induced 6/11 kidney tumours, mainly nephroblastomas, adenomas and clear-cell carcinomas (Ito, 1973). Single injections to Wistar rats of 0.125 mg/rat at 1 day of age and 0.125 or 10 mg/rat at day 7 induced 63% kidney tumours after median induction times of 218-237 days; 92% of the induced neoplasms were stromal nephromas (Campbell *et al.*, 1974).

Hamster: Weekly s.c. injections of 0.5-1.0 mg NDMA for 6-20 weeks (total dose, 6-14 mg/animal) caused haemangioendothelial sarcomas and

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cholangiocarcinomas in the liver and aesthesioneuroepitheliomas of the
nasal cavity in Syrian golden hamsters (Herrold, 1967). Lifetime weekly
injections of approximately 6, 3 and 1.5 mg/kg to groups of 32, 32 and 30
Syrian golden hamsters (males and females) led mainly to liver tumours:
3, 10 and 8 in males, and 4, 4 and 2 in females, respectively. The liver
tumours were: hepatocellular carcinomas (6), hepatomas (8) and cholangio-
carcinomas (17). Only a few tumours were seen in the respiratory tract
(3), the kidney (1) and the forestomach (1) (Haas et al., 1973). Similar
results were obtained in another study (Stenback et al., 1973).

In Chinese hamsters, weekly injections of 0.89, 1.77 or 3.54 mg/kg bw
NDMA for lifetime (total doses, about 32, 54 or 104 mg/kg bw, respectively)
caused mainly liver haemangioendotheliomas (70-100%). Only 3/108 hamsters
had adenocarcinomas in the endoturbinals of the nasal cavity (Reznik, 1975;
Reznik et al., 1976).

In 60 European hamsters, weekly s.c. injections of 1.4-8.6 mg/kg bw
NDMA induced mainly malignant haemangioendotheliomas of the liver and
kidney, hepatocellular carcinomas and 1 cholangiocellular carcinoma. No
such tumours were seen in 20 controls (Mohr et al., 1974).

Mastomys: Twice-weekly s.c. injections of 0.1 mg NDMA/animal for
10-44 weeks induced liver tumours in 6/36 males: 2 cholangiomas, 2 cholan-
giocarcinomas, 1 haemangioendothelioma and 1 hepatic-cell carcinoma;
none were seen in the females. In controls, 4/82 liver tumours were seen
in females and none in males (Fujii & Sato, 1970).

(d)   Intraperitoneal administration

Mouse: A single dose of 7 or 14 mg/kg bw NDMA given to GR or CFW/D
mice resulted in lung adenomas (Den Engelse et al., 1969/1970; Frei, 1970).
Single doses of 5, 10 or 15 mg/kg bw NDMA to RF mice induced 9/18, 16/19
and 4/5 lung tumours (adenomas, papillary carcinomas), as compared with
25/52 in controls. The incidence of leukaemia was not significantly
different from that in controls (Clapp, 1973). Weekly injections of 6 mg/
kg bw for 10 weeks in female Swiss mice resulted in a significantly increased
incidence (P<0.001) of vascular tumours (40%), mainly of the retroperitoneum,
while the incidence in males was only 15%. There was a low incidence of

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hepatic vascular tumours in both sexes (Cardesa *et al.*, 1973). Single doses of 7.5 or 15 mg/kg bw NDMA given to NZO/Bl mice at 60 days of age induced a large increase in the incidence of lung tumours in males and females (76-100%, compared with 19-25% in controls) and of kidney adenomas and carcinomas in males (33% (7/21) in the lower dose group and 56% (10/33) in the higher dose group, compared with only 1/268 in controls) (Noronha, 1975). In adult C3Hf mice, a single dose of 7 mg/animal induced hepatomas in 38% of males but none in females (Den Engelse *et al.*, 1974). A positive dose-response relationship was seen in Swiss mice with regard to lung adenoma incidence: 0 mg/kg (control), 15%; 0.5, 17%; 1.0, 29%; 2.0, 35%; 4.0, 39%; and 8.0, 67%. No clear relationships between applied dose and tumour rate was apparent for other tumour sites and in other strains (ASW/SN and A) (Ii *et al.*, 1976).

Newborn and suckling mouse: Single i.p. injections of 8 mg/kg bw NDMA to newborn mice resulted in hepatomas (hepatocellular type) and lung adenomas (Frei, 1970). Six i.p. injections of 1-4 mg/kg bw to 7-day-old mice resulted in hepatomas, hepatocarcinomas, lung adenomas and haemangiomas (Vesselinovitch, 1969).

Rat: A single i.p. injection of 18 mg/kg bw NDMA to Wistar rats induced kidney tumours (Murphy *et al.*, 1966). In 80-day-old NZR rats, starved for 48 hours, a single i.p. dose of 20 mg/kg induced squamous-cell carcinomas of the nasal cavity (Noronha & Goodall, 1972).

The morphogenesis of kidney tumours induced by i.p. injections of NDMA in rats has been described by Hard & Butler (1971a) and Hard *et al.* (1977).

Newt: While single doses of up to 16 g/kg bw NDMA did not induce tumours, 6-7 injections of 16 g/kg bw within 3-4 weeks induced liver tumours (described as anaplastic and hepatic-cell tumours) in 3 newts surviving more than 3 months (Ingram, 1972).

(e) Other experimental systems

Various injections: Rats given a single injection of 18 mg/kg bw NDMA, either intramuscularly, retroperitoneally, or directly into the kidney, developed kidney tumours (Murphy *et al.*, 1966).

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Prenatal exposure: In mice, single or repeated injections of 12.5-75 mg/kg bw NDMA during the last days of pregnancy resulted in lung adenomas and hepatomas in the offspring (Smetanin, 1971). NDMA induced a low frequency of kidney tumours in the offspring of pregnant rats treated during the last week or during the whole of pregnancy (total dose, 11 mg) (Alexandrov, 1968).

Immersion: In 50 guppies exposed for 7 weeks to 100 mg/l NDMA dissolved in aquarium water, an incidence of 6/44 liver tumours was observed (Khudoley, 1973; Pliss & Khudoley, 1975). In frogs (*Rana temporaria*), 5 mg/l NDMA in tank-water induced 19/43 tumours: hepatocellular carcinomas and adenomas and tumours of the haematopoietic system (Khudoley, 1977).

Carcinogenesis in plants: Seeds of hybrids of *Nicotiana* were soaked in 10 or 1 mM solutions of NDMA in water for 1 or 2 days, and tumours were found on 6.5-12.8% of germinated seedlings 20 days later, as compared with 1-2% on controls (Andersen, 1973).

(f) Carcinogenicity of precursors

Administration of 0.1 or 0.025% aminopyrine together with 0.1 or 0.025% sodium nitrite in the drinking-water of Sprague-Dawley rats for 30 weeks produced haemangioendothelial sarcomas in the liver in 29/30 and 26/30 rats, while 0.1% aminopyrine alone was inactive; 0.1% oxytetracycline and 0.1% sodium nitrite administered in a similar protocol induced 4/30 liver tumours (3 hepatocellular carcinomas and 1 cholangioma) (Taylor & Lijinsky, 1975). NDMA is the known reaction product of both interactions in the animal body (Lijinsky & Greenblatt, 1972).

(g) Carcinogenicity of derivatives

N-Nitroso-*N*-methyl-*N*-acetoxymethylamine

(i) Oral administration

I.g. administration of 3.5 or 1.75 mg/kg bw to Sprague-Dawley rats twice-weekly resulted in squamous-cell carcinomas and papillomas of the forestomach in 13/16 and 18/20 animals, respectively (Wiessler & Schmähl, 1976).

(ii)   Intraperitoneal administration

A single i.p. injection of 13 mg/kg bw N-nitroso-N-methyl-N-acetoxy-methylamine to 4-5-week-old CD rats resulted in a high incidence (70-86%) of tumours, mostly in the intestine (Joshi *et al.*, 1977; Ward *et al.*, 1977).

(h)   Factors that modify carcinogenicity in animals

Feeding of a protein-free diet for 1 week, then a single i.p. injection of 60 mg/kg bw NDMA, and a further week on protein-free diet induced 14/14 kidney tumours in Porton rats, compared with 0/9 in controls given the protein-free diet only (McLean & Magee, 1970). Similar results were obtained by Hilfrich *et al.* (1975).

In rats, the incidence of kidney tumours produced by feeding NDMA at a level of 500 mg/kg of diet for 2 weeks was markedly increased by subsequent treatment with 5000 mg/kg of diet of the nephrotoxin N-(3,5-dichlorophenyl)succinimide; pretreatment with this compound inhibited tumour induction (Ito *et al.*, 1974; Sugihara & Sugihara, 1976). A similar increase in the incidence of renal-cell tumours was produced by subsequent treatment with citrinin (Shinohara *et al.*, 1976).

Tumours of the kidney developed in 33% of male and 63% of female Wistar rats that received a single i.p. dose of 30 mg/kg bw NDMA. I.p. injections of 100, 200 or 300 mg/kg bw ethylmethanesulphonate 8 hours after NDMA injection increased kidney tumour rate, more in females than in males (Montesano *et al.*, 1974). In this experiment, 5/109 heart tumours of the left ventricle were observed in the combined treatment groups and diagnosed as either neurofibromas or neurinomas; 1/34 and 8/118 heart tumours were seen in the groups treated with NDMA or ethylmethanesulphonate, respectively (Haas *et al.*, 1974).

Actinomycin D, given before or after a single i.p. dose of NDMA, did not significantly modify kidney tumour incidence in Sprague-Dawley rats (Hilfrich *et al.*, 1975).

Gonadectomy abolished the kidney tumour response observed in male N20 mice after a single i.p. injection of 7.5 mg/kg bw (Noronha, 1975).

Combined gavage of 0.4 mg NDMA in 1 ml tap-water 5 times weekly for
24 weeks to rats together with 3-methylcholanthrene did not increase the
incidence of liver tumours; however, tumours of the lung and skin were
found (Hoch-Ligeti et al., 1968). Hepatocarcinogenesis induced by 4 mg/
kg bw/week NDMA was effectively suppressed by administration of 500 mg/kg
bw/week disulfiram 2 hours before NDMA treatment but led to the an inci-
dence of 59% of squamous-cell carcinomas in the paranasal sinus; this
tumour type was not observed with NDMA or disulfiram alone (Schmähl et al.,
1976). Administration of a copper-deficient or excess-copper diet at the
same time as treatment with 50 mg/l NDMA in the drinking-water did not
change the incidence of liver or lung tumours; however, kidney neoplasms
occurred in 57% of rats receiving the copper-deficient diet, as compared
with 0% in the excess-copper groups (Carlton & Price, 1973). A diet high
in fat and marginally deficient in lipotropes did not enhance hepatocarcino-
genesis by NDMA, although this effect was seen for other nitrosamines
(Rogers et al., 1974).

Hepatocarcinogenesis in Sprague-Dawley rats after a single i.g. dose
of 20 mg/kg bw NDMA was increased by pretreatment with carbon tetrachloride
42 or 60 hours before oral administration. A high incidence of papillary
adenocarcinomas and nephroblastomas was observed in a group that received
40 mg/kg bw NDMA 42 hours after administration of carbon tetrachloride;
no kidney tumours were seen in the group treated with NDMA only (Pound &
Lawson, 1975; Pound et al., 1973). A single i.p. injection of 12-15.6 mg/
kg bw NDMA to female rats 24 hours after partial hepatectomy induced hepato-
cellular carcinomas; none developed in intact animals receiving NDMA
(Craddock 1973, 1975). An increase in the incidence of NDMA-induced kidney
tumours after partial hepatectomy was also seen (Craddock, 1971; Pound &
Lawson, 1975).

Repeated i.p. injections of phorbol given after a single s.c. injec-
tion of 0.015 mg NDMA increased the incidences of lung and liver tumours
in newborn AKR mice (Armuth & Berenblum, 1972). Cardesa et al. (1974b)
described synergestic effects of i.p. injections of NDMA and N-nitrosodi-
ethylamine; with NDMA alone, lung carcinomas and kidney tumours were seen,

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while with NDMA and *N*-nitrosodiethylamine lung adenomas and forestomach papillomas but no kidney tumours were seen.

Thyroidectomy significantly increased the tumour incidences in kidney and liver in NZR rats given single i.p. injections of 20 mg/kg bw NDMA; it reduced the sex difference in lung tumour incidence (male, 70%, female, 16%; compared with 54% and 39%, respectively) (Noronha & Goodall, 1976).

Treatment of Syrian golden hamsters with 15 mg/l NDMA in drinking-water and with a mixture of three antibiotics (gentamicin, nystatin and vancomycin) increased tumour incidence (liver carcinomas, cholangiocar-cinomas, Kupffer-cell sarcomas) to 80% as compared with an incidence of 28% with NDMA alone in females, and to 21% compared to 4% in males (Love *et al.*, 1977).

Administration of aminoacetonitrile to rats decreased the carcinogenic effects of NDMA; the number of malignant liver tumours induced decreased by 80% (Hadjiolov, 1971).

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

In rats, the acute LD_{50} for NDMA was 40 mg/kg bw when given by oral administration, 37 mg/kg bw by inhalation (Druckrey *et al.*, 1967) and 43 mg/kg bw by i.p. administration (Heath, 1962). The i.p. LD_{50} in mice was 20 mg/kg bw (Frei, 1970). The s.c. LD_{50} was 30 mg/kg bw in Syrian golden hamsters (Haas *et al.*, 1973), 18 mg/kg bw in Chinese hamsters (Reznik, 1975), 43 mg/kg bw in female and 28 mg/kg bw in male European hamsters (Mohr *et al.*, 1974).

The major toxicity in a number of species arises from a severe centri-lobular necrosis in the liver (Magee & Barnes, 1967; Magee & Swann, 1969). Inhibition of protein and nucleic acid synthesis in the liver by NDMA has been reviewed (Magee & Barnes, 1967; Magee & Swann, 1969; Pegg, 1977).

Embryotoxicity and teratogenicity

No teratogenic effects were observed in rats. A single dose of 30 mg/kg bw given intraperitoneally or orally, or 20 mg/kg bw injected intra-venously, caused an increase in foetal mortality, particularly when given on days 3, 9, 10 or 12 of gestation (Napalkov & Alexandrov, 1968). After intraplacental injections of 0.1-0.3 mg NDMA into each embryonic sac on day 13 of gestation, all embryos died (Alexandrov, 1974).

Absorption, distribution and excretion

The uptake of NDMA from the gastrointestinal tract has been studied in rats. Uptake from the stomach was slow, but that from the upper part of the small intestine was very rapid ($t\frac{1}{2}$: less than 5 min); absorption from the lower part of the small intestine and the caecum was slower but still much faster than from the stomach (Hashimoto et al., 1976; Heading et al., 1974). Comparisons of the relative methylation of liver and kidney DNA after small oral or i.v. doses of NDMA in rats have indicated that oral doses below 40 µg/kg bw were completely metabolized by the liver and did not enter the general circulation (Diaz Gomez et al., 1977) [see also 'Metabolism'].

In goats, one hour after oral administration of 30 mg/kg bw, 12.2 mg/kg NDMA were found in milk and 10 mg/kg NDMA in blood. Only traces of NDMA were found in the milk after 24 hours (Juszkiewicz & Kowalski, 1974).

Metabolism

Available evidence suggests that NDMA requires metabolic activation to exert its toxic and carcinogenic effects. The rate of metabolism of NDMA in vivo has been examined by measuring the rate of loss of NDMA from the blood and exhalation of $^{14}CO_2$ following administration of ^{14}C-NDMA. In rats, a dose of 30 mg/kg administered by i.p. injection is metabolized within 6 hours (Heath, 1962; Magee et al., 1976; Phillips et al., 1975; Swann & McLean, 1971). The rate of metabolism of NDMA in vitro has been measured by the use of slices of liver and other organs (e.g., kidney, lung, small intestine, oesophagus) from rats, hamsters, monkeys, trout, goldfish and various amphibians. Labelled $^{14}CO_2$ was produced from ^{14}C-NDMA, and

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methylation of cellular macromolecules was observed (Den Engelse *et al.*, 1975; Montesano & Magee, 1974). These data, together with the distribution of methylated nucleic acids *in vivo* in rats, suggested that the liver is the principal site of NDMA metabolism (Swann & Magee, 1968). Oxidative *N*-demethylation to form formaldehyde has been demonstrated with liver microsomes from rats, mice and hamsters (Argus *et al.*, 1976; Czygan *et al.*, 1973a; Lake *et al.*, 1976; Lotlikar *et al.*, 1975; Mizrahi & Emmelot, 1962). Microsomal oxidation has been suggested to result in an unstable *N*-nitroso-*N*-methyl-*N*-hydroxymethylamine, which decomposes to yield a methylating species and formaldehyde (Druckrey *et al.*, 1967; Magee & Barnes, 1967). This mechanism is consistent with the observation that *N*-nitroso-*N*-methyl-*N*-acetoxymethylamine is hydrolysed to yield an alkylating intermediate (Roller *et al.*, 1975).

Microsome-mediated methylation of both protein and nucleic acid by NDMA has been demonstrated *in vitro* (Chin & Bosmann, 1976; Kim *et al.*, 1977). 7-Methylguanine derived from NDMA-d$_6$ has three deuterium atoms, indicating that it must be formed *via* a methyldiazonium ion or a methyl carbonium ion rather than from diazomethane (Lijinsky *et al.*, 1968).

NDMA treatment has been reported to induce methylation of cellular protein, including histones (Magee & Hultin, 1962; Turberville & Craddock, 1971).

Studies have been made of the alkylation of cellular nucleic acids after NDMA treatment *in vivo* or in tissue slices *in vitro*. The major product was 7-methylguanine, but a number of minor products have also been identified, including 1-methyl-, 3-methyl- and 7-methyladenine, O^6-methyl- and 3-methylguanine, 3-methylcytosine, 3-methyl- and O^4-methylthymine and methyl phospho esters (Lawley, 1974; Magee *et al.*, 1976; Montesano & Magee, 1974; O'Connor *et al.*, 1972, 1973, 1975; Pegg, 1977; Swann & Magee, 1968). Single s.c. injections of ^{14}C-NDMA into pregnant mice on day 12 of gestation produced no alkylation of DNA bases in embryonic tissues, but when given on day 18 of gestation, methylation of foetal liver and brain DNA bases was found. A similar extent of alkylation was obtained when 18-day-old foetuses were treated directly in the same manner (Bochert, 1975).

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Studies have supported the hypothesis of Loveless (1969) that the
formation of O^6-methylguanine may be of critical importance in carcino-
genesis and mutagenesis, since this product is formed in much smaller
amounts by other methylating agents which are less active as carcinogens
or mutagens than NDMA (Lawley, 1974; Magee et al., 1976; Pegg, 1977).

Distribution of methylated bases produced by NDMA may not be entirely
random within DNA of the chromatin (Cooper et al., 1975; Ramanathan
et al., 1976), and it has been demonstrated that hepatic mitochondrial DNA
is alkylated more extensively than nuclear DNA (Wilkinson et al., 1975;
Wunderlich et al., 1971/1972). Alteration in the velocity sedimentation
rate of liver DNA in alkaline sucrose gradient, attributed to single-strand
breaks, occurs in the DNA of rats treated with NDMA (Damjanov et al., 1973).
Repair of single-stranded regions in hepatic DNA following administration
of NDMA, as determined by chromatography on benzoylated DEAE-cellulose, is
more rapid than in kidney DNA (Huang & Stewart, 1977; Stewart & Huang, 1977).

Hepatic DNA damaged by alkylation after NDMA treatment is replicated
(Rajalakshmi & Sarma, 1975). The persistence of O^6-methylguanine in DNA
and the rate of cell division may be important factors in the tissue-specific
induction of tumours by NDMA (Margison et al., 1976; Nicoll et al., 1975;
Pegg, 1977). O^6-Methylguanine is removed from the DNA of liver and kidney
by an enzyme system (Nicoll et al., 1975; Pegg, 1977). There is evidence
that NDMA-induced carcinogenic damage to the kidney can also be repaired
(Swann et al., 1976). Administration of NDMA induces selective prolifera-
tion of tubular epithelium and interstitial cells in rat kidney (Hard, 1975;
Stewart & Magee, 1971).

Administration of aminoacetonitrile to rats reduced the metabolism of
NDMA, the induction of hepatic DNA damage and the acute toxicity (Fiume
et al., 1970; Hadjiolov & Mundt, 1974; Stewart, 1974). Such pretreatment
also reduced the production of a mutagenic metabolite from NDMA by rat liver
microsomes (Bartsch et al., 1975). Pretreatment of rats with pregnenolone-
16α-carbonitrile (PCN) decreased the acute toxicity of NDMA (Somogyi et al.,
1972) but did not greatly reduce either conversion of ^{14}C-NDMA to exhaled
$^{14}CO_2$ or methylation of nucleic acids (Grandjean & Somogyi, 1976). However,

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liver microsomes from PCN-pretreated rats showed an increased ability to convert NDMA to an intermediate mutagenic for *Salmonella typhimurium* G-46 (Bartsch *et al.*, 1975). The acute toxicity of NDMA in rats and mice and the *N*-7-methylation of guanine in liver DNA were reduced by simultaneous administration of disulfiram (Schmähl *et al.*, 1971). Microsomes from disulfiram-treated rats showed a decreased ability to convert NDMA into intermediates mutagenic for *S. typhimurium* G-46; mutagenicity was also reduced when disulfiram was added to the assay systems (Montesano & Bartsch, 1976).

Mutagenicity and other short-term tests

The mutagenic effects of NDMA have been reviewed by Montesano & Bartsch (1976). In the presence of a metabolic activation system, genetic activity has been demonstrated in *S. typhimurium* (reverse mutations), *Escherichia coli* (forward and reverse mutations), *Bacillus subtilis* (reverse mutations), *Saccharomyces cerevisiae* (back mutations, gene recombination and conversion), *Serratia marcescens* (reverse mutations) and *Neurospora crassa* (forward mutations) (Montesano & Bartsch, 1976). A mutagenic agent was produced from NDMA in the presence of mouse kidney microsomes, and there was a correlation between the ability to produce mutations in *S. typhimurium* G-46 and susceptibility for kidney tumours in different strains of mice (Weekes, 1975). NDMA was mutagenic in *S. typhimurium* G-46 in the presence of a microsomal fraction from human liver biopsies (Czygan *et al.*, 1973b).

NDMA has been tested in the host-mediated assay using mice or rats. Mutagenicity was shown in *S. typhimurium* strains G-46, C-207 and C-340 after their i.p. injection into Swiss albino mice that had received 3 i.m. injections of 0.1 ml of a 10% NDMA solution at 1-hour intervals (Gabridge & Legator, 1969). Fahrig (1975) observed mutagenicity in *S. cerevisiae* D-4 injected into the peritoneum, the testes or the tail vein of male Wistar rats injected subcutaneously with 740 mg/kg bw NDMA.

NDMA injected subcutaneously into mice at doses of 30 mg/kg bw or 300 mg/kg bw was mutagenic in a blood-mediated assay in which stationary cells of *E. coli* K-12 were injected intravenously (Mohn & Ellenberger, 1973).

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NDMA and *N*-nitroso-*N*-methyl-*N*-acetoxymethylamine induced X-linked recessive lethal mutations in *Drosophila* (Fahmy *et al.*, 1975; Pasternak, 1962). *N*-Nitroso-*N*-methyl-*N*-acetoxymethylamine was also mutagenic in *S. typhimurium* TA 1530 and in bacteriophages R17 and T7 (Bartsch *et al.*, 1977; Shooter & Wiessler, 1976).

In the presence of a rat liver microsomal fraction from phenobarbital-treated rats, NDMA induced 8-azaguanine-resistant mutants in Chinese hamster V79 cells (Kuroki *et al.*, 1977). Concentrations from 8-135 mM induced chromosome aberrations and sister chromatid exchanges in Chinese hamster cells in the presence of liver microsomal preparations from rats (Natarajan *et al.*, 1976).

Chromosome aberrations, mainly dicentrics and deletions, were found in liver cells of Chinese hamsters injected intraperitoneally with 5 g/kg bw NDMA (Brooks & Cregger, 1973). In the dominant lethal test in mice, an i.p. dose of 9 mg/kg NDMA was ineffective (Epstein *et al.*, 1972), while 4.4 mg/kg administered subcutaneously produced a significant increase in dead implants (Propping *et al.*, 1972). Cultured lymphocytes taken from rats 6 hours after an i.p. injection of 30 mg/kg bw NDMA showed an increased frequency of chromosome aberrations (Lilly *et al.*, 1975).

Unscheduled DNA synthesis has been observed in isolated rat hepatocytes treated with NDMA (Williams, 1976, 1977) and in human fibroblasts in culture in the presence of NDMA and a mouse liver microsomal fraction (Laishes & Stich, 1973).

Rat liver epithelial-like cells maintained in culture were transformed by treatment with NDMA, and the transformed cells induced tumours at the site of injection in 32/42 newborn rats (Montesano *et al.*, 1973, 1975; Williams *et al.*, 1973). Cells cultured from kidneys of rats treated *in vivo* with a single i.p. dose of 60 mg/kg bw NDMA underwent transformation as determined by various *in vitro* criteria (Borland & Hard, 1974; Stewart & Hard, 1977). These transformed cells were described as morphologically similar to the putative precursors of NDMA-induced mesenchymal neoplasms (Hard & Butler, 1971b; Hard & Borland, 1977).

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(b) Humans

In 4 men, laboratory exposure to NDMA gave rise to acute liver necrosis which later developed into cirrhosis; in one case, the acute liver injury proved to be fatal (Barnes & Magee, 1954; Freund, 1937).

Studies *in vitro* suggest that NDMA is metabolized by human liver and lung *via* the same metabolic pathway as in other mammalian species (Harris *et al.*, 1977; Montesano & Magee, 1970).

3.3 Case reports and epidemiological studies

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

N-Nitrosodimethylamine is carcinogenic in all animal species tested: mice, rats, Syrian golden, Chinese and European hamsters, guinea-pigs, rabbits, ducks, mastomys, various fish, newts and frogs. It induces benign and malignant tumours following its administration by various routes, including ingestion and inhalation, in various organs in various species. It produces tumours, mainly of the liver, kidney and respiratory tract. It is carcinogenic following its administration prenatally and in single doses. In several studies, dose-response relationships were established.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group. Available information on occurrence suggests that the general population may be exposed to low levels of *N*-nitrosodimethylamine; however, no exposed group suitable for an epidemiological investigation has yet been identified. Reports of relatively high levels in certain pesticide formulations and of occupational exposures that may have occurred in the manufacture and use of rocket fuels may permit the identification of exposed groups.

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4.3 Evaluation

There is *sufficient evidence* of a carcinogenic effect of *N*-nitrosodi-
methylamine in many experimental animal species. Similarities in its
metabolism by human and rodent tissues have been demonstrated. Although
no epidemiological data were available (and efforts should be directed
toward this end), *N*-nitrosodimethylamine should be regarded for practical
purposes as if it were carcinogenic to humans.

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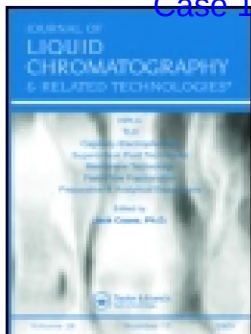
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Exhibit 17

Identification and Control of Impurities for Drug Substance Development using LC/MS and GC/MS

Heewon Lee , Sherry Shen & Nelu Grinberg

Exhibit 0028

Hecht

Identification and Control of Impurities for Drug Substance Development using LC/MS and GC/MS

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Abstract: Identification and control of impurities for drug substances is a critical task in pharmaceutical process development for quality and safety. The most commonly used analytical technique for impurity analysis in drug substances and drug products is undoubtedly a chromatographic method, namely high performance liquid chromatography (HPLC). Impurity profiling is typically performed by HPLC and impurities are further tested for identification and confirmation by other techniques. Several case studies are presented in this paper to report the identification of unknown impurities employing chromatographic techniques interfaced with mass spectrometry. The task of unknown identification was facilitated by complementary methodologies including tandem mass spectrometry (MS/MS), high resolution mass spectrometry (HRMS), preparative HPLC and NMR. Upon identification of the impurity, the impurity formation was monitored and controlled throughout the synthesis. Three case studies are described where unknown process impurities were analyzed for identification using LC/MS and GC/MS methodologies. It is demonstrated that identification of the unknown impurity enabled chemists to pinpoint the chemical step of impurity generation, aiding the effort to reduce or even eliminate the impurity in the drug substances.

Keywords: Control of impurities, Drug development, GC/MS, LC/MS

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INTRODUCTION

Impurity profiling is one of the most critical analytical tasks during the development of drug substances (also known as active pharmaceutical ingredients, API). The level of impurities is tightly controlled by regulatory agencies for toxicological assessment and clinical studies. ICH guideline Q3A(R) requires that organic impurities at or above 0.1% (or 1.0 mg total daily intake, whichever is lower) should be identified for drug substance with maximum daily dose of less than 2 g/day.[1] The most established analytical method for impurity profiling is indisputably high performance liquid chromatography (HPLC) with UV detection. The organic impurities are usually determined by HPLC/UV first and further analyzed by other analytical techniques including LC/MS, MS/MS, HRMS, preparative LC, and NMR (nuclear magnetic resonance). With the advent of atmospheric pressure ionization methods such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) enabling a smooth transition of samples from the liquid phase (HPLC) to the gas phase (MS), LC/MS has become a prominent analytical technique for both quantitative and qualitative purposes in pharmaceutical research and development.[2]

Identification and tracking of organic impurities using LC/MS related technologies for drug substance[3–12] and drug product[13–16] development is well documented in the literature. Most of the reported cases employed multi-disciplinary approaches in order to elucidate the impurity structures, namely, tandem mass spectrometry (MS^n), high resolution mass spectrometry (HRMS), LC/UV, LC/MS, LC/NMR, NMR and preparative LC. Quadrupole-based mass spectrometers (single quadrupole and triple quadrupole MS) are by far the most widely used MS types. Single quadrupole MS can provide molecular ion information and in certain cases fragmentation data through in-source collision induced dissociation (CID). In comparison, triple quadrupole MS is useful for acquisition of MS^2 tandem mass spectral data. It has been reported that trace level impurities were identified using ion trap multistage tandem mass spectrometry followed by preparative LC for confirmation by NMR.[3,10] Ion trap-based mass spectrometry provides MS^n fragmentation pathways, compared to quadrupole-based mass spectrometry which offers MS^2 stages, rendering in-depth structural information for impurity identification.[2] High resolution mass spectral data can add another dimension to the analytical information for the determination of impurity by providing elemental compositions.[11,14,15] The combination of the multistage tandem mass spectrometry and accurate mass measurement gives an extremely powerful analytical tool, FT-ICR-MS (Fourier Transform Ion Cyclotron Resonance Mass Spectrometry). This device

is capable of providing elemental compositions of the molecular and fragment ions for the n-th order of tandem mass spectral data. Several groups reported the use of FT-ICR-MS for the identification and confirmation of unknown impurities.[8–10] The process impurities are expected to have structural similarity to the drug substance. Therefore, the mass spectral fragmentation pattern of the process impurities were compared to that of the drug substance, and impurity structures were postulated in the reports. The identity was subsequently confirmed by NMR experiments on isolated impurities, or in certain cases the postulated compound was synthesized for unequivocal confirmation.

Isolation of the impurity via column chromatography or preparative HPLC is a labor-intensive and time-consuming task, and therefore it is avoided whenever possible. However, there were reported cases where the unexpected toxicity and color of the drug substance made the fractionation of the impurity inevitable. The impurity responsible for the unexpected color of the drug substance was much less than 0.1%, but the impurity had to be identified to understand the origin of formation and to reduce or eliminate the impurity.[4] An unexpected toxic response during an animal toxicological study from a drug substance batch was observed, and the batch was fractionated to pinpoint the impurity, though the level of the impurity was lower than 0.1%.[11] In both cases, preparative HPLC was used to fractionate and enrich the impurities.

Most drug substances are too polar or thermally labile to be subject to a gas chromatographic analysis, which requires vaporization of the samples into the gas phase. However, for analysis of raw material and intermediates, gas chromatography offers several advantages including high separation efficiency, wide dynamic range, and various compatible detectors such as FID (flame ionization detection), ECD (electron capture detection), TCD (thermal conductivity detection), and MS. Especially GC/MS is a powerful analytical technique for identification of unknowns due to the availability of vast and accessible spectral libraries.

In this report, three case studies of unknown process impurities are presented. The impurities were first detected by HPLC/UV and GC/FID, and they were subject to LC/MS and GC/MS analyses to obtain molecular ion information. Further analyses such as MS/MS, HRMS, preparative HPLC, NMR, and synthesis of the authentic compound were performed to elucidate the impurity structures. The knowledge obtained helped to understand impurity formation and was used to improve the quality of the drug substances.

EXPERIMENTAL

Reagents

HPLC grade water and acetonitrile were obtained from EMD Chemicals (San Diego, CA, USA). Formic acid was purchased from Fluka (St. Louis, MO, USA), and perchloric acid was obtained from Sigma Aldrich (St. Louis, MO, USA). All reagents were used as received.

Instrumentation

Agilent (Santa Clara, CA, USA) 1100 HPLC systems with ChemStation software were employed for liquid chromatography work. A typical HPLC system consisted of a degasser, a binary pump, an autosampler, an ALS thermostat, a column compartment, and a diode array detector (DAD). GC/FID and GC/MS data were obtained using Agilent G6890N with FID (flame ionization detection) or MSD (mass selective detection) with ChemStation software. A Micromass/Waters (Milford, MA, USA) Quattro Ultima triple quadrupole mass spectrometer with MassLynx control was used for tandem mass spectrometric data generation. An ionization mode of positive electrospray was used with the following parameters: capillary voltage 3.5 kV, cone voltage 20–35 V, drying gas nitrogen, collision gas argon, and collision cell pressure 1.0×10^{-3} mbar. High resolution mass spectral data were obtained using an Agilent LC/MSD TOF mass spectrometer in a positive electrospray ionization mode with the following parameters: capillary voltage 3.0 kV, drying nitrogen gas temperature 350°C, and fragmentor 70–150 V. The samples were introduced to the mass spectrometer via flow injection without a chromatographic separation. Impurity purification by preparative LC was performed using a Shimadzu (Kyoto, Japan) system consisting of a SIL-10AP auto-injector, two LC-8A pumps, SPD-10A VP UV-VIS, SCL-10A VP system controller, and FRC-10A fraction collectors with Discovery VP software.

Case Study A

Analytical HPLC Conditions

The column used for analytical HPLC experiments was an Agilent Zorbax SB-CN 4.6 mm × 150 mm, with particle size of 3.5 um. Mobile phase A was water with 0.5% (v/v) perchloric acid, and mobile phase B was acetonitrile. The flow rate was 1.5 mL/min with the column

temperature maintained at 25°C. Gradient elution profile was to hold
at 0%B for 3 min, linear gradient to 40%B at 4 min, hold at 40%B
until 12 min, linear gradient to 60%B at 17 min, to 100%B at 18 min,
and hold at 100%B until 20 min. The sample diluent was methanol and
injection volume was 5 µL. UV detection wavelength was 220 nm. The
perchloric acid used in the mobile phase was replaced with formic acid
for acquisition of mass spectral data.

Preparative HPLC Conditions

For preparative purification of the impurity, a Waters XBridge Prep
C18 OBD (19 mm ID × 150 mm L, with 5 µm particle size) was used.
The mobile phase A was water and the mobile phase B was acetonitrile
without any modifier. The use of modifier in the preparative LC was
intentionally avoided whenever possible to prevent any decomposition
of the collected samples, even though it meant a slight loss in the
chromatographic resolution and peak shape. The acidic or basic modifier
used in the LC mobile phase could become concentrated during the
solvent evaporation step, and cause decomposition of the collected
fractions.[5] The flow rate was 10 mL/min with a gradient elution profile
from 10%B to 37%B in 20 min, and constant at 37%B until 50 minutes.
The UV wavelength was at 220 nm and the column was held at an
ambient temperature. The sample diluent used was methanol. The
solvents were evaporated from the fractions using a rotary evaporator.

GC/FID Conditions to Monitor the Intermediate B

The GC column used was DB1701, 30 m length, 320 µm ID, with film
thickness of 1.0 µm. The carrier gas was helium with a constant flow rate
of 1.5 mL/min. The inlet temperature was 250°C with a split of 30:1. The
oven temperature profile was to hold at 80°C for 0.5 min, to ramp to
220°C at a rate of 15°C/min, and to hold at 220°C for 1 minute. The
FID temperature was 300°C.

Case Study B

A Waters XBridge C18 column (4.6 mm × 75 mm, with 2.5 µm particle
size) was used and water (mobile phase A) and acetonitrile (mobile phase
B) with 0.1% formic acid were employed as mobile phases. The flow rate
was 2.0 mL/min with a gradient of 20%B to 90%B in 10 minutes. The
column temperature was kept at 40°C.

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Case Study C

The GC column was Agilent DB1701, 30 m × 0.32 mm ID, with 1 μm
film thickness. Inlet temperature was at 200°C with helium as a carrier
gas, constant pressure at 10 psi, and split of 25:1. The GC oven
temperature profile was to hold at 35°C for 7 minutes, ramp to 80°C at
7°C/min, ramp to 180°C at 15°C/min, then hold at 180°C for 2 minutes.
The FID temperature was 250°C.

Additional experimental conditions are described as needed in the
Results and Discussion section for each example discussed.

RESULTS AND DISCUSSION

Case Study A

An HPLC method was developed for reaction monitoring of a fixed
synthetic route for a development compound. Figure 1(a) shows an

(a)

(b)
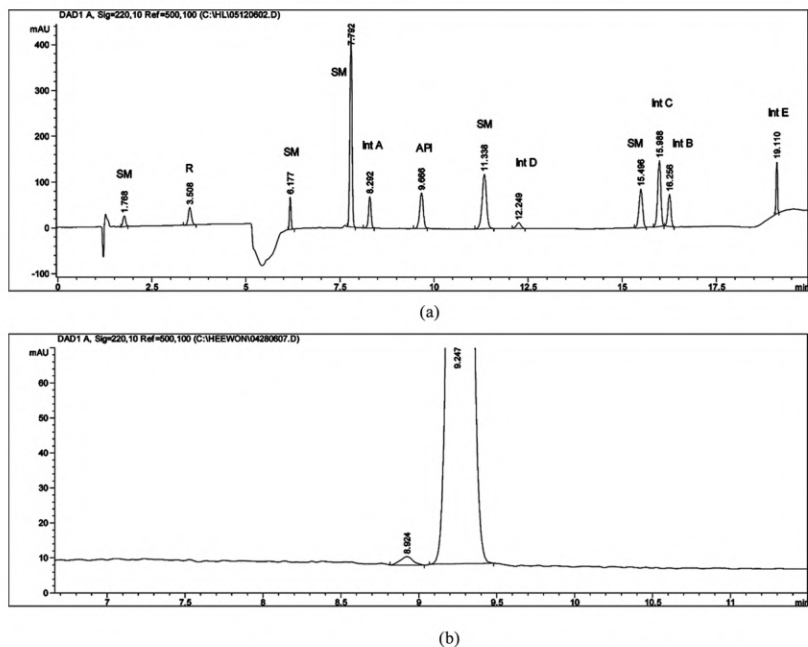
Figure 1. Case Study A: (a) Chromatographic separation of starting materials
(SM), reagents (R), intermediates (Int), and API. (b) The HPLC chromatogram
showing the impurity contained in the first batch of the drug substance.

HPLC chromatogram of the starting materials, reagents, intermediates, and the drug substance (i.e., API). This chromatogram was obtained from an artificial mixture of the involved compounds in order to illustrate the chromatographic separation. The intermediates and API were indicated on the chromatogram. This HPLC method was successfully utilized during the synthetic steps. The synthetic route involved a linear synthesis (intermediates A → B → C → D → E) for the production of API. This method was employed to profile impurities in the first batch of the drug substance. The batch did not have any detectible amount of intermediates, but there was an impurity in the level of 0.6% eluting right before the product shown in Figure 1(b).

In order to identify this unknown impurity, LC/MS experiments were performed. The experimental conditions of the HPLC method were transferred to LC/MS, except for the mobile phase composition. The 0.5% perchloric acid in the mobile phase A of the original HPLC method was substituted with 0.1% formic acid for LC/MS experiments for modifier volatility. This substitution did not affect the selectivity of the impurity from the drug substance considerably.

Figure 2(a) shows the total ion chromatograms (TIC) of the drug substance and the crystallization mother liquor. The retention time of the drug substance was 7.8 minutes and that of the impurity was 7.5 minutes. The mass spectra show the molecular ion for the drug substance and the impurity in Figure 2(b). The molecular ion of the desired product was observed at m/z 396, and the impurity showed an ion at m/z 378 with a mass difference of 18 amu. For tandem mass spectrometric experiments the mother liquor obtained from crystallization was utilized in order to obtain stronger signal intensity of the impurity.

One of the most widely used tandem mass spectrometric techniques using a triple quadrupole MS is the daughter ion (also known as fragment ion) scan. In this scan mode, the first quadrupole is set to pass only the ion of interest, usually the molecular ion. The second quadrupole is used as a collision cell with a controlled amount of collision gas, typically argon. The selected ion from the first quadrupole undergoes collision induced dissociation (CID) in the second quadrupole. The fragment (daughter) ions generated in the second quadrupole are then mass-resolved in the third quadrupole, generating structure-specific fragmentation information.

The MS/MS experiments were performed to obtain daughter ion spectra of the drug substance molecular ion at m/z 396. The fragmentation spectra were acquired at different collision energies (10, 20, 30, 40, 50 and 60 V) in order to cover various fragmentation patterns. The two representative spectra at different collision energies are shown in Figure 3(a). As expected, the molecular ion was still present and a few fragment ions were observed at low collision energies; and at

(a)

(b)
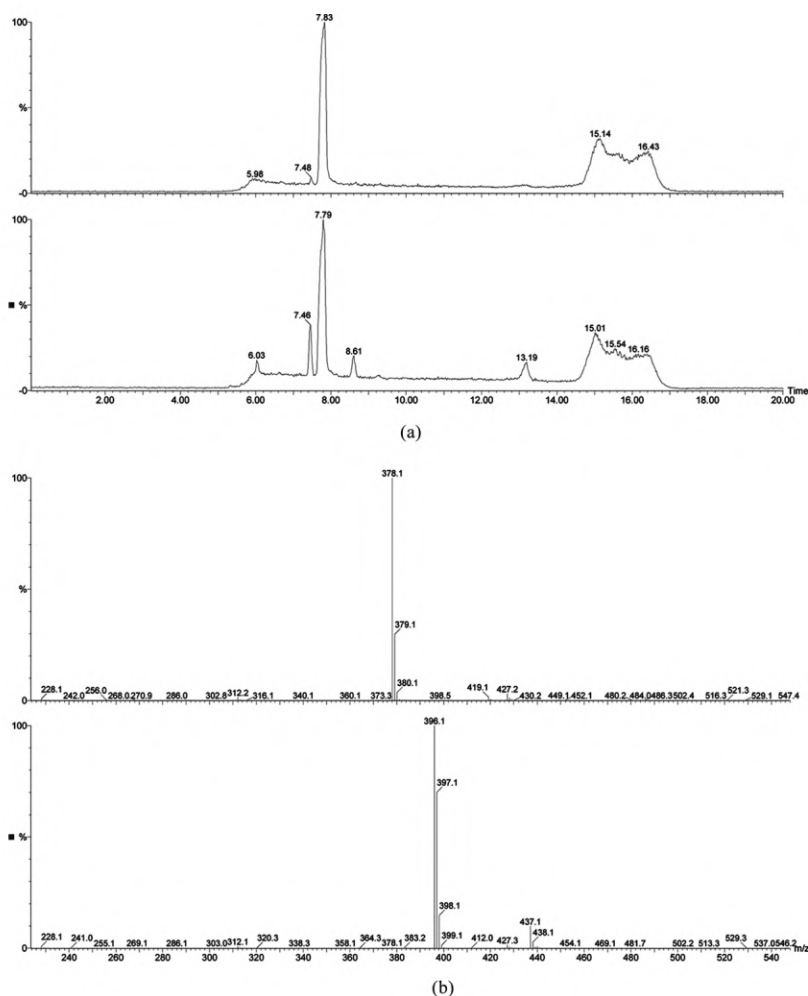
Figure 2. Case Study A: (a) Total ion chromatogram (TIC) of the drug substance (above) and the mother liquor (below). The retention time of the drug substance is 7.8 minutes and that of the impurity is 7.5 minutes. (b) The mass spectra show the molecular ion of the impurity at m/z 378 (above) and the drug substance at m/z 396 (below).

high collision energies the molecular ion disappeared and more extensive and smaller fragment ions were observed. The daughter ion scan of the impurity at m/z 378 showed a similar trend in Figure 3(b).

The illustrative structure of the drug substance is shown below with the key fragments observed in the daughter ion scan data. The mass

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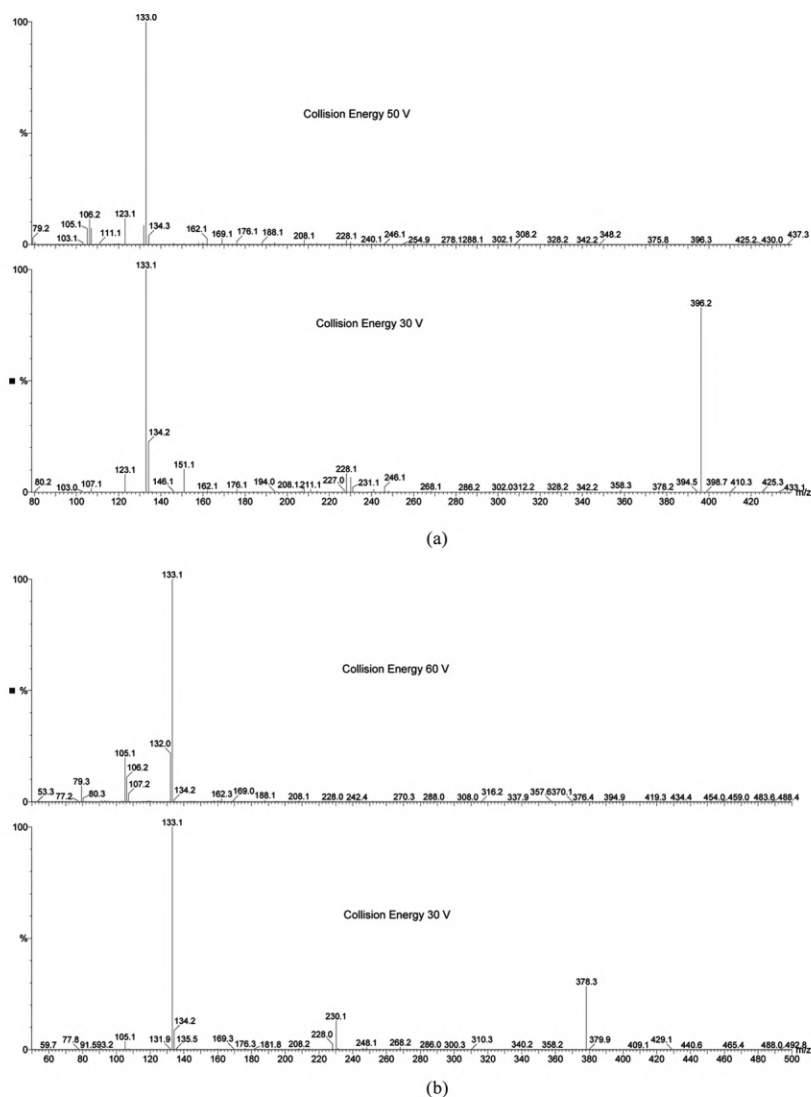
Figure 3. Case Study A: Fragment ion mass spectra at two difference collision energies are shown for the drug substance at m/z 396 (a) and for the impurity at m/z 378 (b).

difference of 18 between the drug substance and the impurity could be the loss of hydroxyl group (–H$_2$O) or the loss of the fluorine atom replaced with a hydrogen (-19 amu for fluorine $+1$ amu for hydrogen $= -18$ amu). Both the drug substance and the impurity had the fragment

ions at m/z 133 and m/z 230, indicating that the right hand side of the
molecule was intact including hydroxyl functional group. However, the
fragment ion at m/z 151 was present only in the drug substance daughter
ion scan, noticeably absent in the daughter ion spectra of the impurity.
Therefore, the difference between the drug substance and the impurity
must have occurred in the left hand side of the molecule. Based on the
data, the impurity was tentatively identified as the des-F (loss or removal
of fluorine) of the drug substance. The ion corresponding to the m/z 151
of the des-F impurity would be m/z 133 (m/z 151–18) that happened to
coincide with the m/z 133 from the right hand side of the molecule.
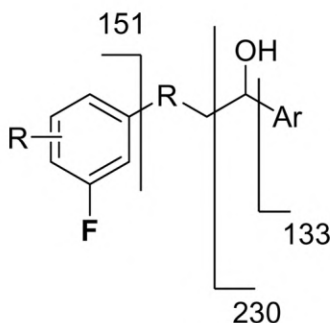
Structure of the drug substance with observed fragments for Case Study A.

The impurity was isolated using a Shimadzu preparative LC system
in order to confirm the identity. The retention time of the impurity was
44 minutes and that of the drug substance was 47 minutes under the
experimental conditions. The fractions collected were subject to high
resolution mass spectrometry (HRMS) using an Agilent 1100 LC and
an Agilent TOF (time-of-flight) mass spectrometer. The HRMS data
showed the correct elemental formula for the drug substance with an
error of 0.1212 ppm. The elemental formula based on the HRMS data of
the impurity fraction matched that of the des-F impurity with an error
of 2.3104 ppm.

The proposed des-F impurity was synthesized to confirm the identity
unequivocally. The NMR data were acquired for both the impurity
collected by preparative LC and the synthesized des-F API, and the
NMR experiments confirmed that they were identical. In an effort to
control the des-F impurity in the drug substance, corresponding des-
F compounds for intermediates A, B, C, and D along the synthetic
route were synthesized. The des-F intermediates A, C, and D were
separated under the existing HPLC conditions as shown in Figure 4(a).
The separation of intermediate B and des-F intermediate B was not ideal

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(a)

(b)
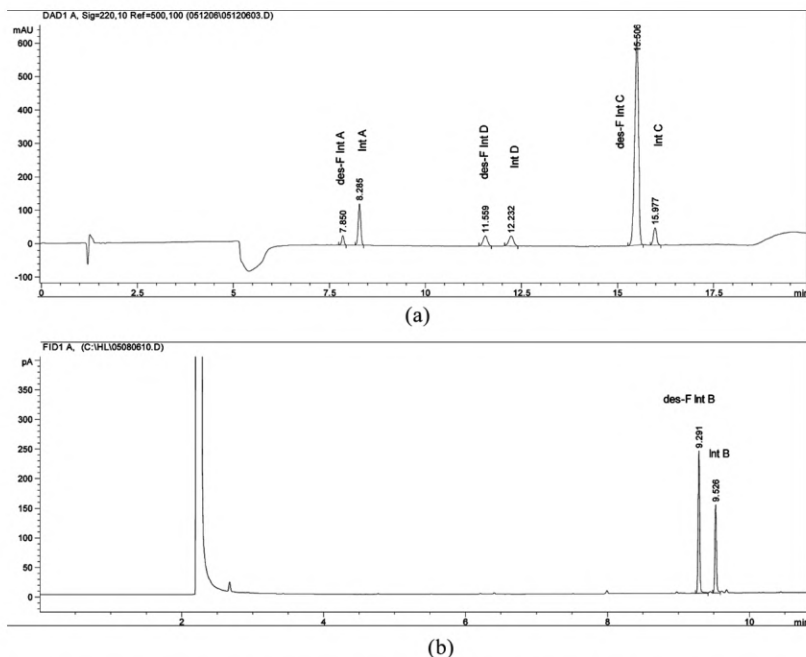
Figure 4. Case Study A: (a) HPLC chromatographic separation of the des-F intermediates A, C, and D from the corresponding intermediates for impurity control. (b) GC/FID method showing the separation of the des-F intermediate B from the intermediate B for impurity control.

in HPLC conditions, thus a GC/FID method was developed to follow the intermediate B as shown in Figure 4(b).

The HPLC/UV and GC/FID methods shown in Figure 4 were employed to carefully monitor the formation of des-F impurities along the synthetic pathway. It was found that the des-F impurity was formed from the chemical step of the intermediate A to the intermediate B, where an equimolar amount of lithium diisopropylamide (LDA) was employed. The formation of the des-F impurity was eliminated by slightly undercharging LDA.

In this case study, the structure of the unknown process impurity was proposed through LC/MS/MS and HRMS. The proposed structure was confirmed by preparative LC, synthesis of proposed compound, and NMR experiments. The origin of the impurity formation was pinpointed, and analytical methods were developed to follow and control the impurity formation throughout the synthesis. We were able to optimize the chemical synthesis so that the process did not generate the impurity.

Case Study B

The drug substance for this project was prepared as a free acid, and subsequently converted to a potassium salt. During the salt formation step a new impurity with an area percent of 2.4% was observed. The molecular ion $[M + H]^+$ for the drug substance was at m/z 642 (Figure 5), with one chlorine indicated by the isotope pattern. The molecular ion of the impurity was at m/z 638, four amu less than the API, clearly missing the isotope pattern of chlorine. As can be seen in the LC/MS data, molecular ions for both the drug substance and the impurity underwent significant in-source collision induced dissociation (CID) generating abundant fragmentation. The major fragment ions observed for the API were m/z 435, 338 and 267, with all three ions showing the pattern of one chlorine. The major fragment ions observed for the impurity were m/z 431, 334, and 263. Interestingly, all three ions were four amu less than the corresponding fragments in the API, and they lacked the chlorine isotope pattern.

In-source CID provided useful fragmentation data for these molecules without the need of a triple quadrupole mass spectrometer. The LC/MS/MS experiments were performed to obtain more detailed fragment ion spectra with a reduced noise level providing higher
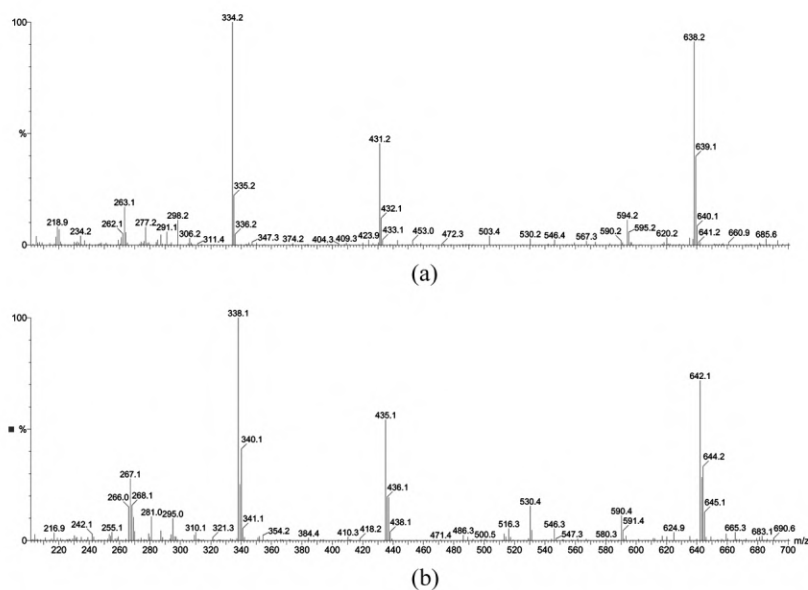
Figure 5.   Case Study B: LC/MS spectra of the drug substance (below) and the impurity (above).

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confidence. The daughter ion scan was carried out at different collision
energies (10, 20, 30, 40, 50, and 60 V) in order to encompass a wide range
of fragmentation. Two representative mass spectra at different collision
energies are shown in Figure 6(a) for the API and in Figure 6(b) for the
impurity. The major ions obtained with LC/MS experiments were still

(a)

(b)
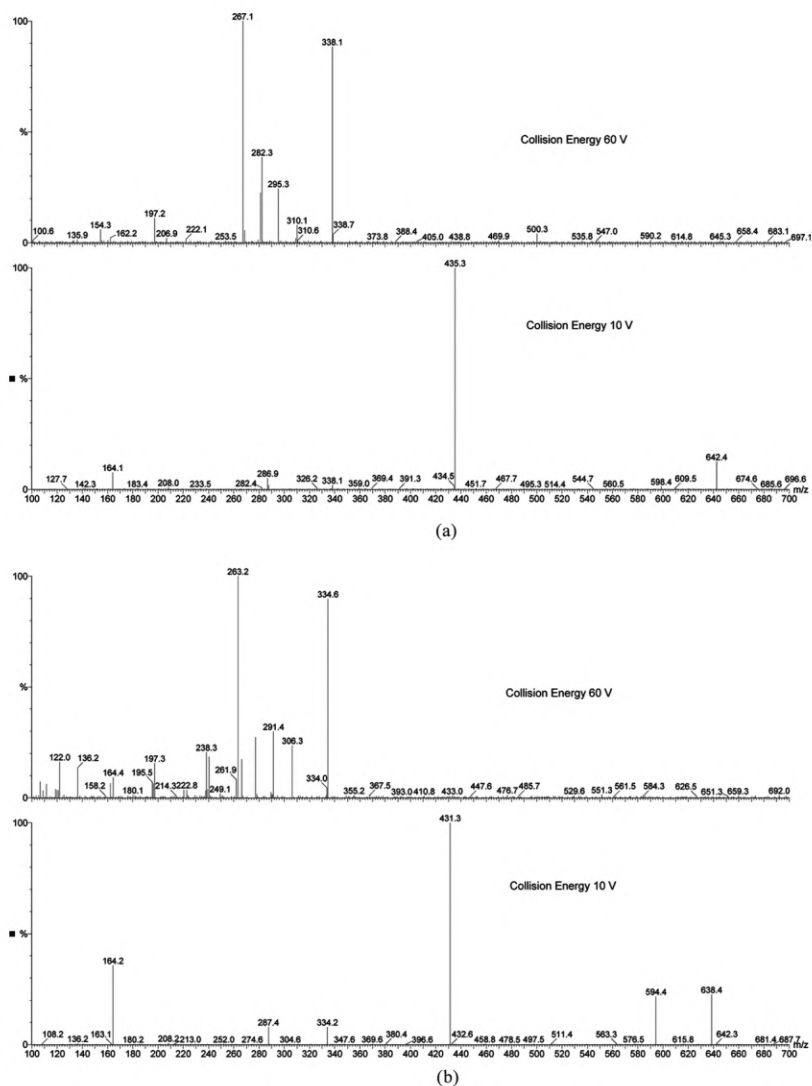
Figure 6. Case Study B: Fragment ion mass spectra at two different collision
energies (a) for the drug substance at m/z 642 and (b) for the impurity at m/z 638.

the major fragments in the LC/MS/MS data including m/z 435, 338 and
267 for the API; m/z 431, 334, and 263 for the impurity. As the first
quadrupole was set at the nominal mass resolution for the LC/MS/MS
experiments, the chlorine isotope was filtered out, therefore, missing the
isotope pattern information. The structure of the API with key fragments
observed is shown below.
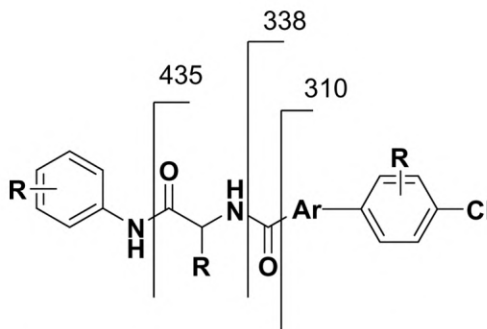
Structure of the drug substance with observed fragments for Case Study B.

There were two obvious pieces of information from the LC/MS
and LC/MS/MS data. The first information was that the impurity was
missing the chlorine, inferred from the molecular ion as well as the
fragment ion isotope patterns. The second information was that all
three major corresponding fragments of the impurity were four amu
less than that of the API, indicating that the modification occurred
on the right hand side of the molecule within the fragment structure
of m/z 310. Since the molecule had to lose the chlorine, the most
straightforward speculation was that "something" replaced the chlorine
on the API. The nominal mass of the major isotope of chlorine is
35, therefore, in order to make the impurity to be 4 amu less than
the API, "something" had to weigh 31 amu. The proposed impurity
structure based on the information was that the chlorine was replaced
with a methoxy ($-OCH_3$, 31 amu = 16 amu for O + 15 amu for CH_3)
group. This made perfect sense as the salt formation was carried out
with methanol as the solvent. The proposed impurity structure was
further supported by HRMS experiments with an error of 2.5237 ppm.
The salt formation step was successfully optimized with regard to the
temperature, reagent addition rate, and reagent addition time in order to
reduce the impurity formation.

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Case Study C

Residual solvent analysis by GC is a routine analytical test performed
for manufacturing of intermediates and drug substances. A GC/FID
method including commonly used solvents was developed for this
purpose and the conditions are described in the experimental section.
One of the process intermediates was analyzed to quantify the residual
amount of tetrahydrofuran (THF). The GC/FID data in Figure 7(a)
showed a peak corresponding to THF at 6.9 minutes. There was a
significant amount of an unexpected impurity present in the intermediate
at a retention time of 13.4 minutes. GC/MS experiments were
performed to acquire mass spectral data for the impurity. The ionization
mode was positive electron impact (EI) ionization at 70 eV. Unlike
electrospray ionization (ESI) which generally produces $[M + H]^+$ ions,
electron impact (EI) ionization produces $M^{+ \cdot}$ radical ions. Electron
impact ionization is a "hard" ionization method producing significant

(a)

(b)
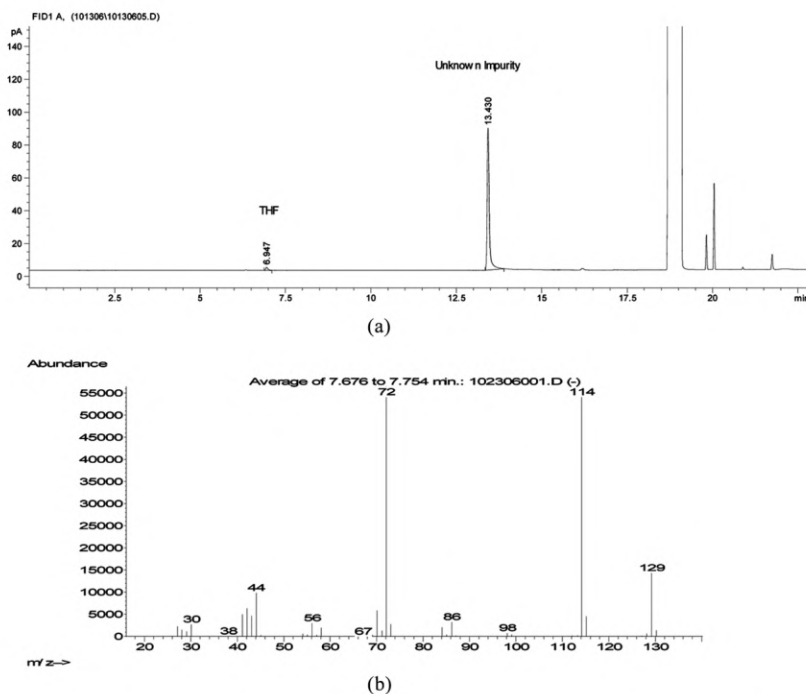
Figure 7. Case Study C: (a) GC/FID chromatogram showing the unknown
impurity. (b) The EI-MS spectrum of the impurity obtained from GC/MS
experiments.

fragmentation of the molecular ions, whereas electrospray ionization is known to be a "soft" ionization technique.[13,17,18] It is relatively easy to compare EI-MS sample data to the library data set and search, mainly due to the following two facts. First, the EI MS data are acquired and cataloged into commercially available libraries at the standardized 70 eV, thus the mass spectra are relatively consistent regardless of differences in manufacturers and instrumentations. Secondly, since it is a hard ionization technique EI-MS yields fragmentation spectra characteristic of the compound.

The mass spectrum acquired for the unknown impurity is shown in Figure 7(b). The library search of the mass spectrum returned diisopropyl ethyl amine (DIEA, also known as Hunig's base) with the highest score. The structure is shown below. The MS spectrum was generated by EI, therefore, the molecular ion observed was at m/z 129, the molecular weight of DIEA. The ion at m/z 114 showed a loss of a methyl group, and the ion at m/z 72 was the result of methyl and isopropyl group loss from the molecular ion. A sample of Hunig's base was analyzed as a standard and the retention time and mass spectral data confirmed the identity of the impurity. Subsequently, appropriate washes were implemented to remove Hunig's base for the preparation of this intermediate.
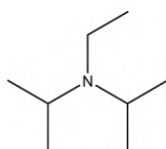
$C_8H_{19}N$
Exact Mass: 129.15
Mol. Wt.: 129.24

Structure of diisopropyl ethyl amine (DIEA).

CONCLUSION

Three case studies of impurity identification and control during the drug substance development were presented. It involved use of several analytical techniques complementing each other, including HPLC/UV, HPLC/MS, tandem mass spectrometry, preparative LC, HRMS, NMR, GC/FID and GC/MS.

Impurity identification of the case study A required a multidisciplinary approach, namely, generation of molecular ion and daughter ions using a range of MS/MS conditions, followed by preparative LC and reference synthesis by synthetic chemists, and

confirmation by HRMS and NMR. Subsequently, analytical methods were developed to follow the impurity throughout the synthetic pathway. In the case study B, the impurity proposed based on the tandem mass spectrometric data was immediately valuable and helped to improve process parameters. The availability of EI MS library for GC/MS made the impurity identification straightforward in the case study C.

It was demonstrated that by identifying the structure of the impurity, the origin of the impurity was pinpointed making elimination/reduction of the impurity achievable. Understanding of the impurity formation expands the knowledge of the process chemistry and enables control of the impurities.

ACKNOWLEDGMENT

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Exhibit 24

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2013 WL 1558690
NOT FOR PUBLICATION
United States District Court, D. New Jersey.

In re FOSAMAX (ALENDRONATE SODIUM)
PRODUCTS LIABILITY LITIGATION.
Bernadette Glynn and Richard Glynn, Plaintiffs,
v.
Merck Sharp & Dohme Corp, Defendant.

Civil Action Nos. 11–5304, 08–08.
|
April 10, 2013.

Attorneys and Law Firms

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Defendant.

OPINION

PISANO, District Judge.

*1 Plaintiffs Bernadette Glynn and Richard Glynn
("Plaintiffs") bring this lawsuit against Defendant Merck,
Sharp, & Dohme Corp. ("Defendant"), which manufactures
Fosamax, a drug approved by the United States Food
and Drug Administration ("FDA") for the treatment and
prevention of osteoporosis. This matter is part of the
multi-district litigation concerning Fosamax and involves
allegations that Fosamax causes atypical femur fractures
("AFFs [1] ") and that it caused Plaintiff Mrs. Glynn
("Mrs.Glynn")'s femur fracture. Presently before the Court
is Defendant's Omnibus *Daubert* Motion to exclude the
expert testimony of Dr. Charles N. Cornell ("Dr.Cornell"),
Dr. Michael J. Klein ("Dr.Klein"), Dr. David Madigan
("Dr.Madigan"), and Dr. Cheryl Blume ("Dr.Blume") as
well as a motion to exclude the causation testimony of
the treating physicians—Dr. Robert Busch ("Dr.Busch"),
Dr. Robert Lindsay ("Dr.Lindsay"), Dr. Frederick Fletcher
("Dr.Fletcher"), and Dr. Britton Limes ("Dr.Limes") [docket

28]. This Court heard oral argument on February 21, 2013
and April 2, 2013. For the reasons outlined below, the Motion
is denied as to Drs. Cornell, Klein, Madigan, and Blume. The
treating physicians' causation testimony will not be excluded
if their opinions are based on their treatment and care of Mrs.
Glynn.

I. DISCUSSION

Federal Rule of Evidence 702 provides that a witness

> qualified as an expert by knowledge, skill, experience,
> training, or education may testify in the form of an opinion
> or otherwise if:
>
> (a) the expert's scientific, technical, or other specialized
> knowledge will help the trier of fact to understand the
> evidence or to determine a fact in issue;
>
> (b) the testimony is based on sufficient facts or data;
>
> (c) the testimony is the product of reliable principles and
> methods; and
>
> (d) the expert has reliably applied the principles and
> methods to the facts of the case.

This Rule requires the proponent of expert testimony to show
the "requisite 'qualifications, reliability, and fit' " or in other
words, that "(1) the witness is qualified as an expert in a
particular field; (2) the methodology applied by the witness is
sufficiently reliable; and (3) the witness's testimony 'fits' the
facts of the case in dispute—that is, the proffered testimony
would assist the trier of fact." *Jones v. Synthes USA Sales,
LLC,* 2010 WL 3311840, *4 (D.N.J. Aug.19, 2010); *see also*
McNamara v. Kmart Corp., 380 Fed. Appx. 148, 151 (3d
Cir.2010); *Meadows v. Anchor Longwall & Rebuild, Inc.,*
306 Fed. Appx. 781, 788 (3d Cir.2009); *Pineda v. Ford
Motor Co.,* 520 F.3d 237, 244 (3d Cir.2008); *Schneider
ex rel. Estate of Schneider v. Fried,* 320 F.3d 396, 404 (3d
Cir.2003).

First, the expert must be qualified; this requirement is
interpreted liberally and "a broad range of knowledge, skills,
and training qualify an expert as such." *In re Paoli R.R.
Yard PCB Litigation,* 35 F.3d 717, 741 (3d Cir.1994).

*2 Second, "an expert's testimony is admissible so long
as the process or technique the expert used in formulating

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the opinion is reliable." *Id.* at 742. An expert's opinion is reliable if it is "based on 'good grounds,' i.e., if it is based on the methods and procedures of science." *Id.* at 744. This inquiry requires a court to examine the "scientific validity and thus the evidentiary relevance and reliability [ ] of the principles that underlie a proposed submission" and to focus "solely on principles and methodology, not on the conclusions ... [the expert] generate[s]." *Daubert v. Merrell Dow Pharmaceuticals, Inc.,* 509 U.S. 579, 594–95, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993). In *Daubert,* the Supreme Court outlined several factors that a court may take into consideration in determining reliability, including whether the hypothesis can be tested, whether the methodology "has been subjected to peer review and publication," the methodology's rate of error, "the existence and maintenance of standards controlling the technique's operation," and whether there is general acceptance in the scientific community. *Id.* at 593–94. The proponent of the expert testimony must demonstrate that the opinions are reliable by a preponderance of the evidence. *In re Paoli,* 35 F.3d at 744.

Third, expert testimony "must fit the issues in the case" or in other words, "be relevant for the purposes of the case and must assist the trier of fact." *Schneider,* 320 F.3d at 404. The Court must determine "whether [the] expert testimony proffered ... is sufficiently tied to the facts of the case that it will aid the jury in resolving a factual dispute." *United States v. Schiff,* 602 F.3d 152, 173 (3d Cir.2010). This standard "is not that high" but "higher than bare relevance." *In re Paoli,* 35 F.3d at 745.

The Court's role, at a *Daubert* hearing, is to act "as a gatekeeper, preventing opinion testimony that does not meet the requirements of qualification, reliability and fit from reaching the jury." *Schneider,* 320 F.3d at 404. In keeping with its gatekeeping role, this Court will apply the *Daubert* analysis to each expert.

A. Dr. Cornell
Plaintiffs offer Dr. Cornell, an orthopedist, as an expert in causation, to establish that Fosamax causes AFFs and Mrs. Glynn's Fosamax use caused her AFF.

1. Dr. Cornell Is Qualified as an Expert
Dr. Cornell is currently a Professor of Clinical Orthopedic Surgery at Weill Cornell College of Medicine and has been the Richard Laskin Chair in Orthopedic Surgery since 2011 [docket # 102, Ex. 8, Dr. Cornell's Report ("Cornell Report") at 2]. In addition, Dr. Cornell is an attending orthopedic surgeon at the Hospital for Special Surgery in New York City and currently serves as the hospital's Director of the Department of Orthopedic Surgery. *Id.* He is a "specialist in orthopedic trauma ... and metabolic bone disease," which includes osteoporosis and osteopenia [docket # 102, Ex. 10, Dr. Cornell's Deposition ("Cornell Dep.") at 69:13–16; 71:14–17]. About 80% of all the fractures Dr. Cornell treats surgically are fractures "as a consequence of osteoporosis or osteopenia." *Id.* at 72:6–21. He has treated two patients with atypical fractures related to bisphosphonate use. Cornell Report at 3. Moreover, he has "participated in a study to determine a management strategy for the treatment of symptomatic bisphosphonate-associated incomplete atypical femoral fractures, which was peer reviewed and published in the Hospital for Special Surgery Journal." *Id.* Although Defendant argues that Dr. Cornell is not qualified because he is not trained in epidemiology and is unfamiliar with "the most basic epidemiological terms and concepts" (Db13[2]), Dr. Cornell does not have to possess a particular subspecialty—epidemiology—to testify as an expert. *See Schneider,* 320 F.3d at 406–07 (determining that testimony was improperly excluded because an individual "was not an expert in the sub-specialty about which he opined"); *Holbrook v. Lykes Bros. S.S. Co., Inc.,* 80 F.3d 777, 783 (3d Cir.1996) (declaring that the lower court erred by requiring the expert to have a particular specialization and "exact background"); *see also Keller v. Feasterville Family Health Care Ctr.,* 557 F.Supp.2d 671, 675 (E.D.Pa.2008) (recognizing that expert testimony cannot be excluded because "the expert is without the appropriate specialization" and that "[a] certain degree of background is not required"). Because Dr. Cornell has the academic background and professional experience with osteoporosis, osteopenia, and fractures associated with those diseases, he is qualified to testify as an expert in this case. *See Schneider,* 320 F.3d at 407.

2. Dr. Cornell's Methodology Is Sufficiently Reliable
 *3 Dr. Cornell formed his opinion using the Bradford Hill criteria, which are "nine factors widely used in the

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scientific community to assess general causation." *Gannon v. United Sates,* 292 Fed. Appx. 170, 173 (3d Cir.2008); Cornell Dep. at 329:5–8. General causation is when "an observed association between a chemical and a disease is causal."

🚩 *Magistrini v. One Hour Martinizing Dry Cleaning,* 180 F.Supp.2d 584, 592 (D.N.J.2002), *aff'd,* 68 Fed. Appx. 356 (3d Cir.2003). The nine Bradford Hill factors are: "1. Temporal Relationship, 2. Strength of the association, 3. Dose-response relationship, 4. Replication of the findings, 5. Biological plausibility (coherence with existing knowledge), 6. Consideration of alternative explanations, 7. Cessation of exposure, 8. Specificity of the association, and 9. Consistency with other knowledge." FEDERAL JUDICIAL CENTER, REFERENCE MANUAL ON SCIENTIFIC EVIDENCE, at 599–600 (3d ed.2011), *available at* http://www.fjc.gov/public/pd f.nsf/lookup/SciMan3D01.pdf/$file/SciMan3D01.pdf; *see also Gannon,* 292 Fed. Appx. at 173 n. 1; *In re Avandia Mktg., Sales Practices & Products Liab. Litig.,* 2011 WL 13576, *3 (E.D.Pa. Jan.4, 2011);

🚩 *Magistrini,* 180 F.Supp.2d at 592–93. "[O]ne or more of the factors may be absent even where a causal relationship exists and ... no factor is a sine qua non of causation."

🚩 *Magistrini,* 180 F.Supp.2d at 593 n. 9.

Dr. Cornell used the Bradford Hill criteria to form an opinion on whether Fosamax causes AFFs. Cornell Dep. at 331:4–8; Cornell Report at 4. In applying the nine Bradford Hill factors, he reviewed Plaintiff's medical records from 1996 to present, the office notes and depositions of her treating physicians, and "past and current medical literature on the topics of osteopenia, osteoporosis and their prevention and treatment with bisphosphonate drugs including alendronate," particularly publications concerning the FIT and FLEX studies and that described the appearance of AFFs. Cornell Report at 3, 4–5. He "review[ed] the original trials, the randomized trials, that led to the approval of Fosamax for the treatment of osteoporosis, and then wanted to review many of the case reports, the case series, the summed analysis, and some of the review papers that took all of this information and put it into a more readily digestible form." Cornell Dep. at 56:13–23. Dr. Cornell attempted to "present a balanced analysis" and pointed out studies on both sides of the issue. *Id.* at 58:5–16. He concluded that Fosamax can cause AFFs and "Fosamax use was a substantial contributing factor to Mrs. Glynn's femur fracture." Cornell Report at 4. The methodology Dr. Cornell used is sufficiently reliable because the Bradford Hill criteria are "broadly accepted" in the scientific community "for evaluating causation," *Gannon,*

292 Fed. Appx. at 173 n. 1, and "are so well established in epidemiological research," *In re Avandia Mktg. ., Sales Practices & Products Liab. Litig.,* 2011 WL 13576, at *3.

*4 Defendant, however, argues that Plaintiffs do not explain the scientific methodology used by Dr. Cornell or show that his methodology is sufficiently reliable. Instead, Defendant asserts that Dr. Cornell's "weight-of-the-evidence" methodology just lists some studies, only some of which support causation, and concludes that the weight of the evidence shows that Fosamax causes AFFs. Defendant explains that this method is inadequate because Dr. Cornell does not discuss how these studies establish causation or why certain studies outweigh others that do not find causation. Additionally, Defendant points out that Dr. Cornell has not done an evaluation of possible biases or confounding factors found in the studies. Because Dr. Cornell does not show that his methodology is sufficiently reliable to show general causation, Defendant argues that he cannot establish specific causation—that Mrs. Glynn's Fosamax use caused her AFF. Defendant explains that the Bradford Hill criteria do not apply to specific causation, and Dr. Cornell's differential diagnosis was unreliable because he did not rule out the possibility that other things could have caused Mrs. Glynn's fracture.

Defendant is free to address these issues on cross-examination, but Defendant's concerns do not prohibit Dr. Cornell from testifying as an expert because he is qualified and the methodology he used is sufficiently reliable. *See*

🚩 *Milward v. Acuity Specialty Products Group, Inc.,* 639 F.3d 11, 15 (1st Cir.2011), *cert. denied,* —— U.S. ——, 132 S.Ct. 1002, 181 L.Ed.2d 734 (2012) (stating *"Daubert* does not require that a party who proffers expert testimony carry the burden of proving to the judge that the expert's assessment of the situation is correct"; instead, the "proponent of the evidence must show only that 'the expert's conclusion has been arrived at in a scientifically sound and methodologically reliable fashion.' ").

Regarding Dr. Cornell's specific causation opinion that Fosamax caused Mrs. Glynn's femur fracture, he applied the differential diagnosis method, which is "a technique that involves assessing causation with respect to a particular individual." 🚩 *Kannankeril v. Terminix Int'l, Inc.,* 128 F.3d 802, 807 (3d Cir.1997). It "is a process by which a physician rules out alternative causes through review of a patient's medical histories and records, physical examination of the patient, laboratory testing, study of

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relevant medical literature, and other techniques." *In re Diet Drugs (Phentermine/Fenfluramine/Dexfenfluramine) Products Liab. Litig.,* 890 F.Supp.2d 552, 561 (E.D.Pa.2012). The "technique is generally accepted in the medical community." *Id.*

Here, Dr. Cornell applied the differential diagnosis method by examining Mrs. Glynn's past medical history and conducting his own examination of her on September 26, 2012, after which he concluded that "[t]o a reasonable degree of medical certainty, Mrs. Glynn suffered a nontraumatic [AFF] in the setting of seven years of full dose Fosamax and alendronate therapy." Cornell Report at 34–36. Dr. Cornell reviewed radiographs taken on April 17, 2009 to evaluate the fracture and reviewed follow-up X-rays, hospital records, rehabilitation records, orthopedics records, prescription records from pharmacies, and deposition transcripts, among other things, in forming his opinion [docket # 109, Ex. 78, Appendix B to Cornell Report]. He ruled out possible alternative causes of Mrs. Glynn's AFF. Cornell Report at 38–40, 42–43, 45–46. Dr. Cornell did not have to "rule out every possible alternative cause of" Mrs. Glynn's AFF; instead, only "[o]bvious alternative causes need to be ruled out." *Heller v. Shaw Indus., Inc.,* 167 F.3d 146, 156 (3d Cir.1999). Thus, Dr. Cornell applied the differential diagnosis method in arriving at his conclusion that Mrs. Glynn's Fosamax use was a substantial contributing factor to her AFF.

*5 Therefore, the methodology used by Dr. Cornell in arriving at both his general and specific causation opinions is sufficiently reliable. Both the Bradford Hill criteria and differential diagnosis are widely used and accepted in the scientific community to arrive at causation opinions.

3. Dr. Cornell's Testimony Fits the Facts of the Case

Finally, Dr. Cornell's testimony fits the facts of the dispute and will assist the trier of fact because Plaintiffs seek to show that Mrs. Glynn's AFF was caused by her Fosamax use and Dr. Cornell not only opines that AFFs are caused by long term bisphosphonate use, like Fosamax, but also that Mrs. Glynn's Fosamax use was a "substantial contributing factor to her" AFF. *See* Cornell Report at p. 22, 47. Consequently, Dr. Cornell's proffered testimony will assist the trier of fact in determining whether Fosamax caused Mrs. Glynn's AFF.

Because Dr. Cornell is qualified, used a methodology that is sufficiently reliable, and his opinion fits the facts of a case, his expert testimony is admissible under *Daubert.*

B. Dr. Klein

Plaintiffs asked Dr. Klein, a pathologist, to offer his opinion on whether Fosamax use causes AFFs and the "mechanism by which those fractures are precipitated" [docket # 103, Ex. 11, Dr. Klein's Report ("Klein Report") at 2].

1. Dr. Klein Is Qualified as an Expert

Dr. Klein is currently the Director of Pathology and Laboratory Medicine at the Hospital for Special Surgery where he has "direct clinical responsibilities for patients ...." *Id.* at 3–4. He also has "direct clinical responsibilities ... as a consultant at Memorial Sloan–Kettering Cancer Center, and as an outside counsel for leading pathology laboratories at major hospitals and institutions around the country." *Id.* at 4. Dr. Klein has reviewed the pathology for at least four patients with AFFs [docket # 105, Ex. 37, Dr. Klein's Deposition ("Klein Dep.") at 41:4–12]. Dr. Klein is currently a Professor of Pathology and Laboratory Medicine at Weill Cornell Medical College. Klein Report at 3. He is involved with several publications, including as the lead author and editor of *Non-neoplastic Diseases of Bones* and *Joints,* the only peer-reviewed, comprehensive textbook on the issue, and as a member of the editorial boards of *Human Pathology, Skeletal Radiology, Advances in Anatomical Pathology,* and *HSS Journal. Id.* Dr. Klein is the Consultant Editor of Research for *The Journal of Bone and Joint Surgery (American)* and has authored or co-authored more than 180 articles, most of which relate to bone pathology. *Id.* Therefore, Dr. Klein possesses "a broad range of knowledge, skills, and training" to qualify him as an expert in pathology. *In re Paoli,* 35 F.3d at 741.

2. Dr. Klein's Methodology Is Sufficiently Reliable

Like Dr. Cornell, Dr. Klein used the Bradford Hill criteria to form his opinion. Klein Report at 2. As discussed above, the Bradford Hill methodology is sufficiently reliable because it is "widely used in the scientific community to assess general causation." *Gannon,* 292 Fed. Appx. at 173. In applying the nine Bradford Hill criteria, Dr. Klein reviewed human and animal studies and studies performed by Defendant to form his opinion. *See* Klein Report at19–38. The studies revealed a strong association between bisphosphonates, like Fosamax, and microdamage in the bones as well as decreased bone toughness. *See id.* at 20, 25–30, 32. In addition, Dr. Klein noted a strong association between delayed fracture healing, due to altered bone quality, in patients and animals taking bisphosphonates. *Id.* at 23–24, 29. These findings

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were replicated in several studies discussed in Dr. Klein's report. Moreover, Dr. Klein cited one study which recognized the *"duration*-dependent, as well as dose-dependent, effect bisphosphonates have on the skeleton." *Id.* at 27. Another study mentioned in Dr. Klein's report noted that the "cessation of bisphosphonate treatment may be prudent for women on therapy who sustain a nonvertebral fracture." *Id.* at 30. Thus, Dr. Klein applied the Bradford Hill criteria, including the strength of association, replication of findings, dose-response relationship, and cessation of exposure factors.

 *6 Based on his review of the studies, Dr. Klein concluded that "alendronate significantly alters the cellular properties of bisphosphonate-treated bone." *Id.* at 38. AFFs are not

> attributed to low bone mass or osteoporosis alone, indicative of bone that has fundamentally compromised bone microstructure. Unless a damaging force exerts tension across the entire cortex, the laws of physics and biomechanics as applied to bone further support the conclusion that bone quality and microstructure must be fundamentally compromised for a transverse fracture in a hollow cylinder[, like the femur,] to follow.

> [*Id.*]

Thus, Dr. Klein opined that there is a causal relationship between Fosamax and AFFs. *Id.* at 2. He used a sufficiently reliable methodology, the Bradford Hill criteria, in forming this opinion.

Defendant, however, argues that the Bradford Hill criteria apply to epidemiology studies, which Dr. Klein's report does not discuss. Defendant contends that Dr. Klein has not provided support for the proposition that a general causation conclusion can be established using the Bradford Hill criteria and human or animal biopsy data. In addition, Defendant asserts that if Dr. Klein discussed epidemiology studies in his report, he did not demonstrate that he is qualified to interpret that evidence because he has no expertise in epidemiology and does not understand the most basic epidemiology terms. Moreover, Defendant points out that Dr. Klein conceded that the mechanism regarding how bisphosphonates cause AFFs has not been established and that the theories Dr. Klein uses to support his conclusion about mechanism—microdamage, decrease in tissue heterogeneity, bone brittleness, and delayed healing—have not been proved with human data.

Yet, Dr. Klein has properly applied the Bradford Hill criteria to epidemiological studies. Epidemiological studies include randomized trials in which one group is exposed to an agent, such as Fosamax, and another group is not, and the effect of the agent or lack thereof is observed. FEDERAL JUDICIAL CENTER, REFERENCE MANUAL ON SCIENTIFIC EVIDENCE at 555–56. Here, Dr. Klein examined randomized trials, such as Dempster et al., Boskey et al., and Donnelly et al.; in each of these studies, some women were given alendronate or another bisphosphonate and others were not. Klein Report at 20–21. Moreover, the Federal Judicial Center's Reference Manual on Scientific Evidence states that "toxicology models based on live animal studies ... may be used to determine toxicity in humans" in addition to observational epidemiology. FEDERAL JUDICIAL CENTER, REFERENCE MANUAL ON SCIENTIFIC EVIDENCE, at 563.

For his testimony to be admissible, Dr. Klein is not required to show that the mechanism has been definitely established. Instead, he just needs to show that the methodology he used to arrive at his opinion is sufficiently reliable. *See* Milward, 639 F.3d at 15 (stating *"Daubert* does not require that a party who proffers expert testimony carry the burden of proving to the judge that the expert's assessment of the situation is correct"; instead, the "proponent of the evidence must show only that 'the expert's conclusion has been arrived at in a scientifically sound and methodologically reliable fashion.' "). Dr. Klein arrived at his opinion on the mechanism by examining several studies and using a scientific method that is sufficiently reliable.

3. Dr. Klein's Testimony Fits the Facts of the Case
 *7 Lastly, Dr. Klein's testimony fits the facts of the dispute and will assist the trier of fact. *See* Jones, 2010 WL 3311840, at *4. Through Dr. Klein's testimony, Plaintiffs seek to show that Fosamax causes AFFs and the mechanism by which this happens. *See* Klein Report at 2. Dr. Klein opines that Fosamax causes AFFs and discusses several ways this happens—microdamage, abnormal osteoclasts, altered bone quality, and delayed fracture healing. Thus, Dr. Klein's testimony will assist the trier of fact in determining whether Fosamax causes AFFs, the ways in which this happens, and ultimately, his testimony will aid the jury in deciding whether Mrs. Glynn's Fosamax use caused her AFF.

C. Dr. Madigan
Plaintiffs asked Dr. Madigan, a statistician, to give his opinion regarding "whether a signal of problematic oversuppression

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of bone turnover and associated [AFF] ... existed for Fosamax, using industry standard pharmacovigilance techniques and data sources, and the adverse event terms selected by Merck to internally evaluate the same" and "assess the strength of that signal, if any, in comparison to the signal, if any, for such events in other products indicated for the prevention and treatment of osteoporosis" [docket # 33, Ex. 30, Dr. Madigan's Report ("Madigan Report") at ¶ 5].

1. Dr. Madigan Is Qualified as an Expert

Dr. Madigan is Professor and Chair of Statistics at Columbia University. *Id.* at ¶ 1. He is an elected Fellow of the Institute of Mathematical Statistics and the American Statistical Association, and from 1995 to 2005 was the 36th most cited mathematician worldwide. *Id.* In 2010, he completed a term as Editor of the journal *Statistical Science. Id.* Dr. Madigan has consulted for companies such as Novartis, Pfizer, and Sanofi–Aventis on several issues, "many related to drug safety." *Id.* at ¶ 2. He has statistical experience with clinical trials and has published more than 100 technical papers on many topics, including pharmacovigilance [3] . *Id.*

Within the last few years, drug safety "with a focus on the development and application of statistical methods for pharmacovigilance" has been "one of [Dr. Madigan's] significant research interests ...." *Id.* at ¶ 3. He has published work in several journals, including *Drug Safety, Pharmacoepidemiology and Drug Safety,* and *Epidemiology. Id.* Dr. Madigan is an investigator in the Mini–Sentinel project, which is "a pilot project sponsored by the FDA to inform and facilitate development of a fully operational active surveillance system, the Sentinel System, for monitoring the safety of FDA-regulated medical products." *Id.* He is the "methods lead for the Observational Medical Outcomes Partnership, a public-private partnership between the FDA and the pharmaceutical industry, which addresses "research methods that are feasible and useful to analyze existing healthcare databases to identify and evaluate safety and benefit issues of drugs already on the market." *Id.* Dr. Madigan is a member of the FDA's Drug Safety and Risk Management Committee, which "advises the FDA Commissioner on risk management, risk communication, and quantitative evaluation of spontaneous reports for drugs for human use and for any other product for which the FDA has regulatory responsibility." *Id.* Dr. Madigan is qualified as an expert because he has "a broad range of knowledge, skills, and training [to] qualify ... [him] as such." 🚩 *In re Paoli,*

35 F.3d at 741. Defendant does not dispute Dr. Madigan's qualifications.

2. Dr. Madigan's Methodology Is Sufficiently Reliable

*8 Dr. Madigan examined the FDA's Adverse Event Reporting System ("AERS") database for a "possible association between Fosamax and a series of ... terms selected by Merck to evaluate oversuppression of bone turnover and associated" AFFs. Madigan Report at ¶ 25. The terms were: bone development abnormal, bone disorder, bone formation decreased, fracture delayed union, fracture malunion, fracture nonunion, low turnover osteopathy, pathological fracture, stress fracture, fracture, and femur fracture. *Id.* at ¶ 26. Dr. Madigan used "two industry-standard signal detection algorithms ... to assess whether or not Fosamax presented a safety signal" indicating oversuppression of bone turnover or AFFs. *Id.* at ¶ 25. The QScan pharmacovigilance software computed the statistics. *Id.* at ¶ 27. Dr. Madigan then compared the Fosamax signals to other oral bisphosphonates and a non-bisphosphonate used for the treatment and prevention of osteoporosis. *Id.* at ¶ 25. After reviewing the data, Dr. Madigan opined that

> industry standard pharmacovigilance techniques and datasources reveal the presence of a clear signal for oversuppression of bone turnover and associated atypical femur fracture events utilizing the terms selected by Merck for such analysis. By standard metrics of "signal" detection, the signal is strong, consistent, and not ambiguous. Of perhaps greater concern, the signal was striking in comparison to that for other drugs indicated for the prevention and treatment of osteoporosis. As early as 2001–2002, the spontaneous report data for Fosamax provide signals for a number of indicators of suppression of bone turnover. For the comparator drugs, such signals either never appear or appear years later.

[*Id.* at ¶ 36.]

This opinion is admissible because it is based on a method that is sufficiently reliable. *See Jones,* 2010 WL 3311840, at *4. Two factors that a court may take into consideration in determining reliability is whether the methodology has been subjected to peer review and publication and whether there is general acceptance in the scientific community. 🚩 *Daubert,* 509 U.S. at 593–94. Here, Dr. Madigan's method, data mining in pharmacovigilance, is generally accepted in the scientific community and has "become routine both in the pharmaceutical industry and amongst regulators worldwide."

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Madigan Report at ¶ 8. In fact, "[p]harmaceutical companies, health authorities, and drug monitoring centers use SRS databases for global screening for signals of new adverse events or changes in the frequency, character, or severity of existing adverse events (AEs) after regulatory authorization for use in clinical practice." *Id.* at ¶ 9. "SRS systems provide the primary data for day-to-day drug safety surveillance by regulators and manufacturers worldwide." *Id.* at ¶ 14. In addition, the QScan software Dr. Madigan used in formulating his opinion is generally accepted by the scientific community because it "has been in widespread use for over 10 years and has been validated extensively." *Id.* at ¶ 28. Moreover, "[m]any peer-reviewed publications report results derived from QScan." *Id.* Thus, Dr. Madigan's methodology is sufficiently reliable.

*9 Although Defendant argues that Dr. Madigan's methodology is unreliable because he did not review the substance of the adverse event reports to see if they actually involve AFFs or oversuppression of bone turnover, this argument is inappropriate on a *Daubert* motion. Dr. Madigan's testimony will be subject to cross-examination, and the credibility of his opinion will be ultimately determined through the adversarial process. Dr. Madigan's methodology is sufficiently reliable because it is generally accepted in the scientific community, and therefore, Plaintiffs have satisfied the second prong of *Daubert.*

3. Dr. Madigan's Testimony Fits the Facts of the Case

Lastly, Dr. Madigan's testimony fits the facts of the case and will assist the trier of fact because it is related to Plaintiffs' failure to warn claim. *See Jones,* 2010 WL 3311840, at *4. A failure to warn claim requires a plaintiff to show "(1) that a manufacturer has a duty to warn (2) against dangers resulting from foreseeable uses about which it knew or should have known and (3) that failure to do so was the proximate cause of the harm." ⚑ *In re Fosamax Prods. Liab. Litig.,* 2013 WL 76140, *3 (S.D.N.Y. Jan.7, 2013). Dr. Madigan's testimony fits the facts of this case because he opines that "[a]s early as 2001–2002, the spontaneous report data for Fosamax provide[d] signals for a number of indicators of suppression of bone turnover," meaning Defendant knew or should have known that Fosamax caused certain dangers in 2001–2002, thus imposing on Defendant a duty to warn of those dangers. Madigan Report at ¶ 36.

Defendant, however, argues that Dr. Madigan's testimony does not fit the facts of the case because it is irrelevant since there is no reasonable standard of care that would have required Defendant to conduct data mining. This is also a matter best left to the credibility determination of the jury.

As a result, Dr. Madigan's expert testimony is admissible under *Daubert* because he is qualified, he used a sufficiently reliable methodology, and his opinion fits the facts of the case.

D. Dr. Blume

Dr. Blume is offered as an expert in pharmacovigilance and FDA regulation. Plaintiffs offer the testimony of Dr. Blume to: (1) "address the timeliness and completeness of the efforts undertaken by [Defendant] ... to fully inform prescribers and patients of the increasingly adverse benefit risk assessments associated with long-term Fosamax use in postmenopausal women"; (2) "evaluate the negative consequences of protracted bone oversuppression," including AFFs, in people receiving Fosamax; and (3) "to consider the pharmacovigilance activities undertaken by [Defendant] to evaluate the noted adverse events during the relevant time periods" [docket # 119, Ex. 33, Dr. Blume's Report ("Blume Report") at ¶ 6].

1. Dr. Blume is Qualified as an Expert

Dr. Blume received her Ph.D. in Pharmacology and Toxicology from the West Virginia University Medical Center and is currently the President of Pharmaceutical Development Group, Inc. (PDG), "a consulting firm ... specializing in pharmaceutical development and registration activities." *Id.* at ¶ 1. In this role, she "has been responsible for preclinical and clinical (Phases I–IV) programs associated with pharmaceutical product development and the securing of pre-marketing approvals" for many drugs before the FDA. *Id.* at ¶ 2. Additionally, Dr. Blume has directed "all phases of interactions with [the] FDA relating to the prosecution of New Drug Applications (NDAs), Abbreviated New Drug Applications (ANDAs), Supplements to New Drug Applications (sNDAs), and the associated approval procedures," including "the collection and evaluation of postmarketing adverse medical events, the preparation of updated product labeling, and the dissemination of accurate, complete and timely product-related information to health care providers." *Id.* at ¶ 3. She was responsible for "regulatory review of promotional and education materials for both brand-name and generic drug products." *Id.* Dr. Blume's responsibilities include the "design, execution, and interpretation of pivotal safety-related trials and the development and implementation of pharmacovigilance

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procedures intended to detect new safety signals and track the evolution of previously identified signals." *Id.* at ¶ 4. She has directed "all phases of interactions with the FDA relating to post-approval labeling procedures regarding changes to safety-related information based upon postmarketing signal tracking and pharmacovigilance efforts," including "collection and evaluation of postmarketing adverse medical events, review and interpretation of the results of postmarketing clinical studies, the preparation of updated product labeling and other communication tools, and the dissemination of new product information to health care providers, patients, and consumers." *Id.* at ¶ 5. Dr. Blume possesses the knowledge, skills, and training necessary to qualify her as an expert. *See* 🚩 *In re Paoli,* 35 F.3d at 741. Defendant does not dispute Dr. Blume's qualifications.

2. Dr. Blume's Methodology Is Sufficiently Reliable

*10 Dr. Blume reviewed published studies (Blume Report at ¶¶ 57–74), Merck's Period Safety Update Reports (*id.* at ¶ 75), Dr. Madigan's report (*id.* at ¶¶ 76–78), Merck's Worldwide Adverse Experience System ("WAES") (*id.* at ¶ 79), and epidemiological studies (*id.* at ¶¶ 82–90). *See also* docket # 119, Ex. 5, Dr. Blume's Deposition ("Blume Dep.") at 148:9–18; 338:9–20 (stating that she looked at the WAES database, literature reports, epidemiological studies, the AERS database, and Dr. Madigan's report). She discussed the "specific regulatory procedures and regulations" pharmaceutical manufacturers have to comply with, including procedures and regulations related to FDA approval, labeling, postmarketing surveillance, and reporting requirements. *Id.* at ¶¶ 11–34. Dr. Blume evaluated all of this information using "her years of experience" in "the industry," *see* 🚩 *In re Viagra Products Liability Litigation,* 658 F.Supp.2d 950, 962 (D.Minn.2009), and opined that

> the scientific literature, Merck's internal adverse event database, the AERS database, and epidemiology analyses confirmed the increasingly adverse risk-benefit profile related to long-term Fosamax use in the indicated populations. However, Merck permitted their labeling and other prescriber information to remain static with respect to both the deteriorating risk-benefit assessment and the escalation in ... [AFF] reports. Such omissions do not comply with the regulatory and industry standards of responsible pharmaceutical companies .... Merck also should have undertaken timely and adequate studies to more clearly elucidate the risks of Fosamax use in the various indicated populations. Finally, Merck should

have disseminated Dear Healthcare Professional Letters to advise prescribers and their patients of the escalating safety and efficacy concerns. Merck's omissions have likely resulted in the exposure of numerous patient populations to unnecessary risks associated with the initiation and ongoing treatment with Fosamax.

[Blume Report at ¶ 110.]

Dr. Blume states that "[b]y the early 2000's, it was known that ... [AFFs] were clinically significant events ...." *Id.* at ¶ 109. Dr. Blume opines that Defendant should have changed the Fosamax label "to include escalating warning and precautionary risk information related to" AFFs. *Id.* Instead, Dr. Blume notes that Defendant "did not identify these fractures in the labeling until 2009" even though it received reports that AFFs were "associated with Fosamax use as early as 2002." *Id.* at ¶¶ 31, 82.

Defendant argues that the Court should exclude Dr. Blume's opinions on: (1) the legal requirements governing pharmaceutical manufacturers and Defendant's compliance with those requirements; (2) Defendant waiting too long to add information about femur fractures to the Adverse Reactions section of the label; (3) Defendant failing to add a warning or precaution about femur fractures to the Fosamax label before 2009; (4) Defendant's failure to timely investigate a potential link between Fosamax and AFF; (5) Defendant's alleged motives or state of mind; (6) the causation or mechanism of AFF; and (7) the drug Evista is safer than Fosamax. Yet, because *Daubert* concerns the narrow issue of whether expert testimony is admissible, this is not the appropriate time for Defendant to request that the Court preclude Dr. Blume from testifying about certain topics. Defendant may question Dr. Blume's opinions or methodology on cross-examination. *See* 🚩 *Milward,* 639 F.3d at 15 (stating "[s]o long as an expert's scientific testimony rests upon "good grounds," based on what is known, ..., it should be tested by the adversarial process, rather than excluded").

*11 Despite Defendant's issues with Dr. Blume's opinions, Plaintiffs have satisfied the second prong of *Daubert* because Dr. Blume's methodology is sufficiently reliable.

3. Dr. Blume's Testimony Fits the Facts of the Case

Dr. Blume's testimony fits the facts of the case because she opines that it was known in the early 2000's that AFFs were associated with Fosamax use. *See* Blume Report at ¶¶ 31, 82.

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Dr. Blume's testimony is relevant and will assist the trier of fact in deciding Plaintiffs' failure to warn claim because Dr. Blume's opinion is relevant to whether and when Defendant knew or should have known that AFFs were associated with Fosamax and therefore, when Defendant should have sought a label change. *See* Schneider, 320 F.3d at 404 (recognizing that expert testimony must "be relevant for the purposes of the case and must assist the trier of fact").

E. Treating Physicians

Defendant argues that the Court should preclude causation testimony from Plaintiffs' treating physicians—Drs. Busch, Lindsay, Fletcher, and Limes—because: (1) Plaintiffs have not provided Rule 26 disclosures for any of the treating physicians; and (2) none of the treating physicians are able to offer a reliable causation opinion to a reasonable degree of medical certainty. Plaintiffs, however, assert that they do not intend to elicit expert testimony from the treating physicians; instead, the treating physicians will testify about Mrs. Glynn's care and treatment, which does not require Rule 26 disclosures.

Treating "physicians are not required to submit expert reports when testifying based on their examination, diagnosis and treatment of a patient." *Patterson v. Howard,* 2010 WL 1050052, *4 (D.N.J. Mar.18, 2010). Federal Rule of Civil Procedure 26(a)(2)(B) requires a witness to submit a written report only "if the witness is one retained or specially employed to provide expert testimony in the case or one whose duties as the party's employee regularly involve giving expert testimony." A "treating physician is not necessarily retained or specially employed to provide expert testimony simply because he or she proffers on causation and prognosis" because "doctors may need to determine the cause of an injury in order to treat it." *Pease v. Lycoming Engines,* 2012 WL 162551, *12 (M.D.Pa. Jan.19, 2012). In order to "determine whether a party retained or specially employed a treating physician to provide expert testimony," the Court must examine "whether the treating physician acquired his opinion as to the cause of ... plaintiff's injuries directly through his treatment of the plaintiff." *Id.* (internal quotation omitted). As a result, treating physicians are not required to submit expert reports "if they form their opinion on causation or prognosis as part of the ordinary care of a patient." *Id.*

Therefore, the testimony of Drs. Busch, Lindsay, Fletcher, and Limes is appropriate if it is based on their care and treatment of Mrs. Glynn. This Court will not allow, however, any expert testimony on causation from these physicians.

II. CONCLUSION

*12 For the reasons outlined above, this Court denies Defendant's *Daubert* Motion as to Drs. Cornell, Klein, Madigan, and Blume. An appropriate Order accompanies this Opinion.

All Citations

Not Reported in F.Supp.2d, 2013 WL 1558690, 91 Fed. R. Evid. Serv. 106

Footnotes

1   The abbreviation of atypical femur fracture (singular) is "AFF."

2   Db13 means page 13 of Defendant's brief.

3   Pharmacovigilance is the surveillance of spontaneous reporting system ("SRS") databases "for the early detection of drug hazards that are novel by virtue of their clinical nature, severity, and/or frequency." *Id.* at ¶ 7.

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Exhibit 25

2006 WL 166452

2006 WL 166452
Only the Westlaw citation is currently available.
NOT FOR PUBLICATION
United States District Court, D. New Jersey.

Jeff PLAYER, et al., Plaintiffs,

v.

MOTIVA ENTERPRISES LLC, a successor
in interest to Star Enterprises, Defendant.

No. Civ. 02–3216(RBK).
|
Jan. 20, 2006.

Attorneys and Law Firms

Keith A. McKenna, McKenna, Mulcahy & McKenna,
Montclair, NJ, for Plaintiffs.

Jeffrey W. Moryan, Connell Foley LLP, Roseland, NJ, for
Defendant.

OPINION

KUGLER, United States District Judge:

 *1 This matter comes before the Court upon motions
by Defendant Motiva Enterprises, LLC, ("Defendant" or
"Motiva") for summary judgment of the claims of Plaintiffs
Jeff Player, et al. ("Plaintiffs"), and to exclude Plaintiffs'
experts Michael Gochfeld, M.D., Ph.D. ("Gochfeld"), R.
Brian Ellwood, Ph.D. ("Ellwood"), Bruce M. Gallo ("Gallo"),
and Daniel McDonald ("McDonald"). For the reasons set
forth below, Defendant's motions will be granted in part and
denied in part.

I. Background[1]
This environmental contamination suit is brought by the
current and former owners of twenty-seven parcels of
residential property located in the Spring Hollow Subdivision
in Gloucester Township, New Jersey.[2] Plaintiffs allege
that emissions from Defendant's nearby Texaco gasoline
service station contaminated their property and the Kirkwood
Cohansey Aquifer, the underground water source for their
potable wells.

Contamination of the aquifer was first detected on April
5, 2000, when significant concentrations of the gasoline-
related compound methyl tertiary butyl ether ("MTBE")
was discovered in a drinking fountain at Camden County
Community College. New Jersey Consumers Water Company
("Consumers"), the entity responsible for providing water
to the college, conducted sampling of some of its wells
and discovered significant amounts of gasoline-related
compounds in municipal supply well number 8 ("CW–8").
Consumers took the well offline on April 10.

While investigating the contamination, the New Jersey
Department of Environmental Protection ("NJDEP")
detected a discharge of volatile organic compounds ("VOCs")
from Defendant's service station, located at 585 Berlin Cross
Keys Road ("Motiva site" or "contamination site").[3] The
NJDEP issued a Field Directive on April 12, 2000, requiring
Motiva to investigate the source and extent of the discharge,
to implement an interim treatment system, and to submit a
remedial action work plan to the NJDEP. Defendant installed
an interim recovery system and twenty-five monitoring and
recovery wells between April and June 2000.

The NJDEP issued a second directive on May 5, 2000,
ordering Defendant to cease gasoline retail operations and
provide treatment or an alternate source of water to replace
CW–8. Defendant replaced the interim system with a
permanent ground water recovery and treatment system in
June 2000, and installed forty-one additional monitoring
wells from June 2000 to present. As further required by the
NJDEP, Defendant regularly sampled potable wells located
on approximately forty residential properties in the vicinity of
the Motiva site. Defendant detected small amounts of MTBE
in thirteen of the residential wells it sampled.[4]

Per the NJDEP directive, Motiva submitted a Remedial
Investigation Work Plan/Remedial Investigation report on
July 2000 and a Remedial Action Workplan ("RAW") on
November 14, 2000. In its RAW, Defendant requested
permission to cease sampling of the residential wells,
contending that the MTBE detected in those wells could not
have come from the Motiva site since the wells are located
upgradient[5] or sidegradient from the site, and no emissions
were detected in most of the monitoring wells between the
Motiva site and the potable wells.[6] Motiva also claimed that
recent literature indicated that traces of MTBE in groundwater
could likely result from "non-point sources." (March 2001
Directive at 2.)

 *2 Plaintiffs' expert, R. Brian Ellwood, Ph.D ("Ellwood"), submitted a response to the RAW on January 17, 2001. In his report, Ellwood notes that as of January 17, 2001, "[c]ontrol of contamination at depth beneath the site, control of offsite contamination, and possibly control of contamination at the northern site boundary, has not been established." (Preliminary Report Sicklerville Road Groundwater Contamination ("Ellwood Report"), McKenna Cert. in Opp. to Def.'s Mot. Summ. J., filed Oct. 12, 2005 ("McKenna Cert."), Ex. F, at 2.) Ellwood also offered possible theories to demonstrate the plausibility of Defendant's responsibility for the MTBE in spite of Motiva's arguments to the contrary.

The NJDEP ultimately rejected Defendant's request to cease sampling of the residential wells in its March 2001 Directive on the basis that "there is insufficient evidence for Equiva to conclude that the MTBE detected in the 13 potable wells in the area did not originate from the Cross Keys Texaco site" and "that regardless of the source of the MTBE in these wells, which is obviously debatable, ongoing sampling of these wells is required *primarily due to their proximity to the site."* (March 2001 Directive at 2) (emphasis in original).

Also in the March 2001 Directive, the NJDEP approved a Classification Exemption Area ("CEA") for the site that excluded all but 1/10 of an acre of 583 Berlin Cross Keys Road (the Wallace Property). The CEA establishes the boundaries of a ground water plume where VOCs exceed the GWQS. [7]

Through summer 2004, the NJDEP regularly reduced the testing requirements. By August 18, 2003, the NJDEP required only:

> annual sampling of the wells at 4, 7, 11, 13 and 14 Donna Marie Court; 2, 4, 6, and 8 Latham Way; 12 and 20 Spring Hollow Drive, and; 937 and 948 Sicklerville Road. For all the sampling events of the aforementioned potable wells conducted April 2002, the Department notes that all wells continue to exhibit no gasoline related contamination in

> excess of the Department's Drinking Water Quality Standards.

(NJDEP Directive, Aug. 18, 2003, McKenna Cert., Ex. D.)

The NJDEP approved shut down of the recovery and treatment system on April 30, 2004. (NJDEP Correspondence, Aug. 9, 2004, Mairo Cert., Ex. S., at 2.) Finally, on August 9, 2004, the NJDEP determined that "Defendant's Remedial Action Progress Reports "meet the conditions of the March 21, 2001 Remedial Action Workplan (RAW) approval. Shell Oil Products U.S. (Shell OPUS) is, therefore, in compliance with N.J.A.C. 7:14B–6." (Aug. 9, 2004, NJDEP Correspondence, Mairo Cert., Ex. S., at 1.)

B. The Residential Properties

Plaintiffs own twenty-seven respective residential properties near Defendant's gasoline station. [8] Twenty-six of the twenty-seven properties-all but 583 Berlin Cross Keys Road ("the Wallace property")-contain potable wells located in the Kirkwood Cohansey Aquifer. Because Plaintiffs' properties are north/northeast of the contamination site, (Undisputed Facts ¶ 38), they are considered upgradient or sidegradient of the contamination site, depending on whether CW–8 is pumping. [9]

 *3 Consistent with the requirements of the NJDEP directives, Defendant tested the Plaintiffs' residential wells for six gasoline-related compounds: benzene, toluene, ethylbenzene, xylenes, MTBE, and TBA. No testing detected any gasoline-related compound on eighteen of the properties. [10] Detection of compounds on the remaining eight properties was as follows:

- A single detection of 0.79 ppb toluene and ten detections of MTBE (highest at 15.5 ppb) at 4 Latham Way,

  - Three detections of MTBE (highest at 0.76 ppb) at 14 Donna Marie Court,

  - Three detections of MTBE (highest at 1.4 ppb) at 6 Latham Way,

  - A single detection of 1.4 ppb toluene at 850 Sicklerville Road,

- A single detection of 0.4 ppb MTBE at 4 Donna Marie Court,

- A single detection of 0.3 ppb MTBE at 12 Donna Marie Court,

- A single detection of 1.2 ppb MTBE at 8 Latham Way, and

- A single detection of 0.3 ppb MTBE at 20 Spring Hollow Road.

The GWQS for toluene is 1,000 ppb and the GWQS for MTBE is 70 ppb. No gasoline-related compound was detected on any Plaintiff's property after April 2001. According to the Certification of Julian Davies, a Project Manager for EnviroTrac, Ltd., an environmental consulting firm retained by Defendant to remediate the Motiva site, the NJDEP never restricted the consumption of water from Plaintiffs' potable wells, and never required Defendant to treat the water, provide Plaintiffs with an alternate source of water, or collect soil samples from the residential properties. [11] (Julian Davies Cert., Mairo Cert., Ex. R, at 2.)

Since the fact of the contamination became known, several Plaintiffs have sold their property. Maria and John Wallace sold 583 Berlin Cross Keys Road for $350,000.00 in September 2001, Plaintiffs Thomas and Tina Stankiewicz sold 9 Spring Hollow Drive in July 2002 for $143,000.00, Barbara Tanner sold 17 Spring Hollow Drive for $134,000.000 in February 2002, Daniel and Maria Rodriguez sold 18 Spring Hollow Drive for $138,000.00 in July 2003, David Lodi sold 5 Donna Marie Court for $104,000.00 in September 2001, 13 Donna Marie Court was sold for $109,900.00 in July 2000, and 19 Spring Hollow Drive was sold for $133,900.00 in May 2001.

Defendant filed motions for summary judgment and to exclude experts on June 24, 2005, after requesting and receiving permission from this Court to extend by one week the date for the filing of dispositive and *in limine* motions. Briefs in opposition were due July 22, 2005, however, Plaintiffs instead filed an untimely request for an extension on August 2, 2005, and a second request on September 6, 2005, moving the deadline to September 30, 2005. On October 5, 2005, Plaintiffs filed another untimely request for an extension, and ultimately did not submit a complete Opposition until October 14, 2005. Nevertheless, because a district court should not grant a

motion for summary judgment without examining the merits, *Stackhouse v. Mazurkiewicz,* 951 F.2d 29, 30 (3d Cir.1991) (citing *Anchorage Assoc. v. Virgin Islands Bd. of Tax Rev.,* 922 F.2d 168 (3d Cir.1990)), this Court will exercise its discretion to consider Plaintiffs' Opposition, even though it is untimely. Local Civ. R. 7.1(d)(5).

II. Standard

*4 Summary judgment is appropriate where the Court is satisfied that "there is no genuine issue as to any material fact and that the moving party is entitled to a judgment as a matter of law." Fed.R.Civ.P. 56(c); *Celotex Corp. v. Catrett,* 477 U.S. 317, 330, 106 S.Ct. 2548, 91 L.Ed.2d 265 (1986). A genuine issue of material fact exists only if "the evidence is such that a reasonable jury could find for the nonmoving party." *Anderson v. Liberty Lobby, Inc.,* 477 U.S. 242, 248, 106 S.Ct. 2505, 91 L.Ed.2d 202 (1986).

The burden of establishing the nonexistence of a "genuine issue" is on the party moving for summary judgment. *Celotex,* 477 U.S. at 330. The moving party may satisfy this burden by either (1) submitting affirmative evidence that negates an essential element of the nonmoving party's claim; or (2) demonstrating to the Court that the nonmoving party's evidence is insufficient to establish an essential element of the nonmoving party's case. *Id.* at 331.

Once the moving party satisfies this initial burden, the nonmoving party "must set forth specific facts showing that there is a genuine issue for trial." Fed.R.Civ.P. 56(e). To do so, the nonmoving party must "do more than simply show that there is some metaphysical doubt as to material facts." *Matsushita Elec. Indus. Co. v. Zenith Radio Corp.,* 475 U.S. 574, 586, 106 S.Ct. 1348, 89 L.Ed.2d 538 (1986). Rather, to survive summary judgment, the nonmoving party must "make a showing sufficient to establish the existence of [every] element essential to that party's case, and on which that party will bear the burden of proof at trial." *Serbin,* 96 F.3d at 69 n. 2 (quoting *Celotex,* 477 U.S. at 322); *Heffron v. Adamar of N.J., Inc.,* 270 F.Supp.2d 562, 568–69 (D.N.J.2003). "If the non-movant's evidence on any essential element of the claims asserted is merely 'colorable' or is 'not significantly probative,' the court must enter summary judgment in favor of the moving party."

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Heffron, 270 F.Supp.2d at 69 (citing *Anderson,* 477 U.S. at 249–50).

III. Motion to Exclude Expert Daniel McDonald

Defendant moves to exclude the testimony of Plaintiffs' expert Daniel McDonald ("McDonald") on the grounds that he is unqualified and his report is unreliable. [12] Admissibility of expert testimony is governed by Federal Rule of Evidence 702 and the United States Supreme Court's decision in *Daubert v. Merrell Dow Pharmaceuticals, Inc.,* 509 U.S. 579, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993). [13] In the Third Circuit, the admissibility of expert testimony is contingent on the "qualifications" of the expert and the "reliability" of his methodology. *In re Paoli R.R. Yard PCB Litig.,* 35 F.3d 717 (3d Cir.1994) (interpreting *Daubert*); *see also Oddi v. Ford Motor Co.,* 234 F.3d 136, 145 (3d Cir.2000).

A. *In Limine* Hearing

In certain instances, courts are obligated to provide *in limine* hearings before applying *Daubert* to exclude expert testimony. *Padillas v. Stork–Gamco, Inc.,* 186 F.3d 412 (3d Cir.1999). A hearing is required, for example, where the court excludes an expert's conclusions on the grounds that they are "insufficiently explained and the reasons and foundations for them inadequately and perhaps confusingly explicated." *Id.* In other words, where a report is "conclusory and did not adequately explain the basis for [the expert's] opinion or the methodology employed in reaching his conclusions," the "plaintiff needs an 'opportunity to be heard' on the critical issues of scientific reliability and validity." *Oddi,* 234 F.3d 136, 152 (3d Cir.2000) (holding that the district court did not err "in granting summary judgment here without an *in limine* hearing") (quoting *Padillas,* 186 F.3d at 417). Where the evidentiary record is substantial, however, or the court has before it the information necessary to determine that the expert lacks "good grounds" for his conclusions, an *in limine* hearing may be unnecessary. *Id.* at 153.

*5 The evidence before this Court clearly establishes the process by which McDonald "arrived at his conclusions," *Oddi,* 234 F.3d at 152, and McDonald's report and deposition details the methodology underlying his determinations. As discussed below, this Court will exclude McDonald's testimony on the grounds that his analysis and methodology are baseless and inconclusive, not because his report is insufficiently explained. Additionally, Defendant's motion for summary judgment alerted Plaintiffs to the *Daubert* challenge, yet Plaintiffs neither requested a hearing nor offered any affidavit or evidence in support of McDonald. Accordingly, an *in limine* hearing is unnecessary.

B. Qualifications

The Third Circuit instructs courts to "liberally" evaluate an expert's qualifications. *Oddi v. Ford Motor Co.,* 234 F.3d 136, 145 (3d Cir.2000). In particular, the Circuit has "eschewed overly rigorous requirements of expertise and [has] been satisfied with more generalized qualifications." *In re Paoli,* 35 F.3d at 741 (citing *Hammond v. International Harvester Co.,* 691 F.2d 646, 652–53 (3d Cir.1982) and *Knight v. Otis Elevator Co.,* 596 F.2d 84, 87–88 (3d Cir.1979)). This liberal treatment extends to the expert's substantive qualifications as well as his formal qualifications. *Id.*

Nevertheless, the Third Circuit has "also set a floor with respect to an expert witness's qualifications." *Elcock v. Kmart Corp.,* 233 F.3d 734, 742 (3d Cir.2000). To demonstrate when an expert would not be qualified under Rule 702, the *Elcock* Court offered the pre-*Daubert* case, *Aloe Coal Co. v. Clark Equip. Co.,* 816 F.2d 110 (3d Cir.1987), which held a tractor salesperson unqualified to testify as an expert about the cause of a tractor fire. *Elcock,* 233 F.3d at 742 (citing *Aloe Coal,* 816 F.2d 110).

In *Elcock* itself, the Court determined with "misgivings" that the district court had not abused its discretion by concluding that a psychologist with experience in obtaining employment for disabled individuals was qualified to testify to the possibility for vocational rehabilitation of the injured plaintiff. However, the Court acknowledged that it also would have upheld a decision to exclude the expert since "he seems most qualified to testify on a micro-level regarding the ability of a disabled individual to return to a specific job; he does not appear particularly qualified to testify on the macro-level regarding the number of jobs in the national or local economy that the disabled individual is able to perform." [14] *Elcock,* 233 F.3d at 744. Taken together, *Elcock* and *Aloe Coal* indicate that where a proposed expert's area of experience is

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adjacent to, but not actually encompassing, the subject matter of his testimony, he may be deemed unqualified.

McDonald has worked as a licensed appraiser in New Jersey for approximately twenty-two years. Defendant argues that McDonald is nevertheless unqualified to testify to the diminution in value of Plaintiffs' properties because McDonald has no experience in appraising contaminated property. Defendant notes that McDonald has never appraised property allegedly contaminated by emissions from a gasoline station and has never acted as an expert in a situation involving contamination of the groundwater or allegations of a leaking underground storage tank. (Daniel McDonald Dep. ("McDonald Dep."), Mairo Cert. in Supp. Def.'s Mot. to Exclude Plaintiffs' Expert Daniel McDonald, Ex. C, at 23–24.) Defendant also points out that McDonald did not entirely understand the Ellwood and Gallo reports upon which he relied, including the charts indicating the presence and degree of contaminating agents on the property. (McDonald Dep. at 55–56.)

*6 This case lies squarely between *Aloe Coal* and *Elcock.* Although McDonald is an experienced appraiser, no evidence indicates that he has any experience appraising contaminated properties or is qualified to value the effects of stigma on property values. Just as a psychologist experienced in assisting individuals to find work may be unqualified to testify about the general availability of jobs in the economy, an individual able to appraise an uncontaminated property may have no grounds for appreciating the devaluation of the same property under unique conditions of contamination or stigma. Because nothing in McDonald's experience indicates knowledge or expertise in issues of contamination, he is unqualified to testify to the loss of value to Plaintiffs' properties arising from the alleged contamination.

C. Reliability

Because expert testimony has the potential to bear considerable weight with a jury, the district court functions as a gatekeeper responsible for assuring "that the scientific methodology upon which the expert opinion is founded is reliable" and that "the expert's conclusion is based on good grounds." *In re Paoli,* 35 F.3d at 732–33. To ascertain "reliability," the court must examine a number of factors, both those established in *Daubert* and those previously enumerated by the Third Circuit in *United States v. Downing,* 753 F.2d

1224 (3d Cir.1985). *Oddi,* 234 F.3d 145 (citing *Paoli II,* 35 F.3d at 742). In particular, the court must consider:

> (1) whether a method consists of a testable hypothesis; (2) whether the method has been subjected to peer review; (3) the known or potential rate of error; (4) the existence and maintenance of standards controlling the technique's operation; (5) whether the method is generally accepted; (6) the relationship of the technique to methods which have been established to be reliable; (7) the qualifications of the expert witness testifying based on the methodology; and (8) the non-judicial uses to which the method has been put.

Paoli II, 35 F.3d at 742 n. 8; *see also Elcock,* 233 F.3d at 746 (noting that "each factor need not be applied in every case"). The party wishing to introduce the testimony bears the burden of establishing "by a preponderance of the evidence that their opinions are reliable." *Paoli,* 35 F.3d at 744.

Of course, an expert's opinion need not be "perfect," and judges may not substitute their opinions for those of an expert. *Paoli,* 35 F.3d at 744; *see also Crowley v. Chait,* 322 F.Supp.2d 530, 536 (D.N.J.2004). However, courts also need not admit mere conclusions or "opinion evidence that is connected to existing data only by the *ipse dixit* of the expert. A court may conclude that there is simply too great an analytical gap between the data and the opinion proffered." *Magistrini v. One Hour Martinizing Dry Cleaning,* 180 F.Supp.2d 584, 608 (D.N.J.2002) (quoting *General Elec. Co. v. Joiner,* 522 U.S. 136, 145–46, 118 S.Ct. 512, 139 L.Ed.2d 508 (1997)).

*7 Mere assumptions, without causal evidence or methodological analysis may be inadmissible. *In re TMI Litig.,* 193 F.3d 613, 667–68 (3d Cir.1999). Conclusions based only on the expert's experience, *Oddi,* 234 F.3d at 140–41, and testimony founded on methods that are not

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generally accepted or lack testable hypotheses may also fail to surmount the *Daubert* standard, 🚩*Elcock,* 233 F.3d at 746. Furthermore, conclusions based on analogies that are too dissimilar to the subject of the testimony may also merit exclusion. 🚩*General Elec.,* 522 U.S. at 144 (rejecting expert testimony that plaintiff's cancer was due to exposure to PCBs when the testimony was based on animal studies of infant mice that had developed cancer after exposure to PCBs).

In response to Defendant's motion to exclude McDonald's testimony, Plaintiffs argue that "Mr. McDonald's opinions are based upon credible facts, NJDEP records, the reports of Plaintiffs' liability experts and individual appraisal reports prepared for each residential property." (Pl.s' Opp. Def.'s Mot. Summ. J. ("Opp."), filed Oct. 12, 2005, at 30.) However, McDonald testified in his deposition that he relied only on the Gallo and Ellwood Reports, and he specifically testifies that he did *not* "review any correspondence from the NJDEP related to this site." (McDonald Dep. at 15.) [15]

In spite of Plaintiffs' arguments to the contrary, this Court cannot avoid the conclusion that McDonald's methodology is entirely unreliable. In his report, McDonald determines that the value of Plaintiffs' properties with no evidence of contamination should be discounted 35% percent and property with onsite contamination should be discounted by 66%. (McDonald Report ("McDonald Report"), Mairo Cert. in Support of Def.'s Mot. Exclude Pl.s' Expert Daniel McDonald, Ex. B., at 31, 33.) McDonald reached the 35% and 66% figures without discussing, or even recognizing, the extent to which the property was actually contaminated. As demonstrated by his ignorance of the "ND"/Not Detected signifier in the Gallo and Ellwood Reports, McDonald did not know how to read the charts denoting the levels of contamination. (McDonald Dep. at 56.) Nor had McDonald ever conducted any physical inspection of or visit to the properties prior to writing the report. [16] (McDonald Dep. at 15–16.)

Furthermore, to quantify the stigma attached to Plaintiffs' properties, McDonald relies upon a highly misleading analogy with a site of profoundly contaminated residential properties in Dover Township. (McDonald Report at 27.) Specifically, McDonald compares Plaintiffs' properties with "an area of Dover Township that had ground water contamination from Union Carbide and ... Ciba Geigy that resulted in what was commonly known as a cancer cluster among children," meaning "an inordinate number of children

with cancer." (McDonald Dep. at 158–59.) McDonald selected the Dover site not because of its comparability, but because McDonald "didn't know of any other cases that, where the data was as readily available." (McDonald Dep. at 159.)

*8 Employing the Dover analogy, McDonald determined that the property in the Dover site is in the final stages of recovery and continues to suffer a stigma loss of 13%. Because McDonald considered Plaintiffs' properties in the early stages of recovery, McDonald determined that they must bear a stigma discount of at least two or three times that of the Dover site, resulting in a discount of 35%. [17] However, the severity of the contamination and resulting illness among Dover residents undercuts any grounds for comparison with Plaintiffs' properties where there were few detections of contaminants and no reported physiological effects.

The methodology employed to reach the 66% figure is equally unreliable. To assess the value of properties with some evidence of contamination, McDonald sent an email to thirteen financial lenders to determine whether they would "lend on a property that has known contamination, or the stigma of contamination, to the ground water." (McDonald Report at 32.) Of the thirteen lenders, six replied. One of those refused to comment, and one said that it would loan given certain circumstances. The other four lenders stated that they would not lend on a property that is contaminated, but the content of their brief responses suggested that they understood the email hypothetical to denote property that was actually contaminated and out of compliance with state requirements. [18]

From the results of the email test, McDonald concludes that there would be no buyers other than those who could pay cash. [19] McDonald then assessed the discount in value given cash-only buyers, extrapolating from this a discount of 66%. (McDonald Report at 33.) However, the reliability of the 66% figure is entirely invalidated by the overemphasis placed on the four responses to the email hypothetical, the misleading implication in the email hypothetical, suggesting a much greater contamination of the property than actually present, and the unclear calculations and assumptions underlying McDonald's arrival at 66%.

Ultimately, McDonald's report does not fulfil any of the reliability factors. His method is untestable and arbitrary, without a generally accepted, established, or peer reviewed methodology, and his evaluation was conducted without any

real standards. Because McDonald is unqualified and his evaluation is unreliable, Defendant's motion *in limine* to exclude his testimony will be granted.

IV. Plaintiffs' Claims

A. Negligence and Gross Negligence

To surmount a motion for summary judgment of a negligence claim, Plaintiffs must provide evidence such that a reasonable jury could find "breach of a duty of care and actual damages sustained as a proximate cause of the breach." *Muise v. GPU, Inc.,* 371 N.J.Super. 13, 35, 851 A.2d 799 (App.Div.2004) (citing *Weinberg v. Dinger,* 106 N.J. 469, 484, 524 A.2d 366 (1987)); *Nappe v. Anschelewitz, Barr, Ansell & Bonello,* 97 N.J. 37, 45, 477 A.2d 1224 (1984) ("[T]he plaintiff must show a breach of duty and resulting damage to prevail in a negligence action."). Motiva argues that Plaintiffs have failed to establish damages and causation and requests summary judgment of Plaintiffs' gross negligence claim on the same basis. [20]

 *9 The absence of an injury will preclude a negligence claim, even where a clear breach of duty is present. *Rocci v. MacDonald–Cartier,* 323 N.J.Super. 18, 24–25, 731 A.2d 1205 (App.Div.1999) (affirming summary judgment for insufficient evidence of damages in defamation case and noting that "a plaintiff must present proof of a material question of fact as to both liability and damages") (citing *Norwood Easthill Assoc. v. Norwood Easthill Watch,* 222 N.J.Super. 378, 384, 536 A.2d 1317 (App.Div.1988) (affirming summary judgment of malicious interference claim on basis that "plaintiff has suffered no injury or damage")). At the summary judgment stage, Plaintiffs must provide actual evidence of injury and cannot simply rely upon "unsubstantiated allegations." *Trap Rock Indus., Inc. v. Local 825,* 982 F.2d 884, 890 (3d Cir.1992) (reversing district court's denial of summary judgment). Just as "a residential customer not in residence during a power loss, or a commercial customer whose store was closed, might have no damages except the inconvenience of resetting clocks," *Muise,* 371 N.J.Super. at 49, 851 A.2d 799, the release of contaminants into the groundwater aquifer does not itself generate damages, unless Plaintiffs can show that they suffered harm.

Plaintiffs concede that they "have not presented and will not present claims for the present manifested bodily injury." (Undisputed Facts ¶ 67.) However, they argue that they have adequately established damages for medical monitoring and property damage. They do not address their claim for emotional distress. [21]

1. Medical Monitoring

Damages for medical monitoring are appropriate where a plaintiff exhibits no physical injury, but nevertheless requires medical testing as a proximate result of a defendant's negligent conduct. *Ayers v. Jackson Twp.,* 106 N.J. 557, 600, 525 A.2d 287 (1987). The risk of injury need not be quantified to merit medical surveillance damages; however, the plaintiff must establish that the risk of serious disease is "significant." *Id.* at 599–600, 525 A.2d 287; *Campo v. Tama,* 133 N.J. 123, 131, 627 A.2d 135 (1993) (awarding medical monitoring damages to a plaintiff with a "fifty- to seventy-five-percent chance of suffering a recurrence of cancer" due to the delay resulting from defendant doctor's malpractice). In the case of toxic exposure, "medical-surveillance damages may be awarded only if a plaintiff reasonably shows that medical surveillance is required because the exposure caused a distinctive increased risk of future injury." *Theer v. Philip Carey Co.,* 133 N.J. 610, 627, 628 A.2d 724 (1993). Such damages are "not available for plaintiffs who have not experienced direct and hence discrete exposure to a toxic substance and who have not suffered an injury or condition resulting from that exposure." *Id.* at 628, 628 A.2d 724.

Low level contamination, "that is, contamination below the minimum level set by DEP for water remediation," typically is insufficient to establish injurious toxic exposure. *Muralo Co., Inc. v. Employers Ins. of Wausau,* 334 N.J.Super. 282, 290–291, 759 A.2d 348 (App.Div.2000) ("[S]ince it is clear that no untreated groundwater is ever entirely pure, we are satisfied that DEP standards are the most reliable guide for determining whether contamination causing damage ... has occurred."). Here, contaminants have been detected in only eight of Plaintiffs' wells, and no detection has been even close to the GWQS. The NJDEP never restricted Plaintiffs' use of water from their potable wells, nor required Defendant to treat Plaintiffs' wells or to provide Plaintiffs with an alternate water source.

 *10 Plaintiffs rely on the testimony of Dr. Michael Gochfeld, Ph.D. ("Gochfeld"), to establish the significant health risks

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and necessity of medical surveillance following from the alleged contamination of Plaintiffs' property. However, nothing in Gochfeld's report concludes that the individual Plaintiffs themselves require medical monitoring under the circumstances. Rather, Gochfeld's report creates a medical monitoring program for a hypothetical target population without taking into consideration the actual exposure of any plaintiff. [22] (Gochfeld Dep. at 26–29.) Gochfeld prepared his report under the assumption that "there were known or actual or potential exposure to a variety of constituents of gasoline." (Gochfeld Dep. at 12.) He states in deposition that he had "no specific factual knowledge of the actual exposures in this case," and he confirms that he has never examined the individual Plaintiffs. (Gochfeld Dep. at 10, 29.)

Gochfeld himself notes that "[w]hether a person exposed to MTBE requires medical monitoring depends in large measure on the level of exposure and the time over which it occurred" and notes that "clearly people that are exposed to MTBE casually would not require one." (Gochfeld Dep. at 24.) Furthermore, Gochfeld stated that he "probably would not" recommend medical monitoring for the minor and often single detections of MTBE on Plaintiffs' properties. [23] (Gochfeld Dep. at 46–50.) Consequently, Gochfeld's report does not establish that Plaintiffs require medical monitoring.

Plaintiffs also appear to argue that their wells may have been more contaminated prior to the initiation of Defendant's testing in July 2000. (Opp. at 20.) However, Plaintiffs provide no evidence suggesting that such exposure actually occurred or that any exposure prior to July 2000 was more than minimal. Plaintiffs also argue for the first time in their Opposition that they may have ingested water from contaminated sources besides the potable wells on their property. (Opp. at 20.) However, Plaintiffs offer no evidence that any Plaintiff actually consumed water from CW–8. Without any evidence supporting their theories, Plaintiffs cannot establish a claim for medical monitoring sufficient to survive summary judgment.

Because Plaintiffs have provided no evidence of a "distinctive increased risk of future injury" from the exposure, Plaintiffs are not entitled to damages for medical monitoring.

2. Property Damage
Defendant requests summary judgment of Plaintiffs' claims of property damage on the grounds that the contamination caused no actual damage to Plaintiffs' properties. [24] Instead of

claiming that their property was physically harmed, Plaintiffs contend that the news of the contamination stigmatized their property, reducing its value in the minds of potential buyers.

In support of their claim for stigma damages, Plaintiffs offer the expert testimony of Daniel McDonald. However, as discussed previously, McDonald's testimony must be excluded as unreliable. Plaintiffs also argue that the testimony of individual Plaintiffs establishes a stigma discount to their property:

> *11 Plaintiff Marie Wallace has submitted sworn Interrogatory statements documenting a $150,000.00 loss on the sale of her property. See Exhibit 0 to McKenna Certification. Other Plaintiffs have similarly provided certified answers to Interrogatories and Deposition testimony as to the loss in value through sales transactions, which occurred from the discharge. See Exhibit N–R to the McKenna Certification.

(Opp. at 20–21.)

This evidence fails to establish an injury. Exhibits N–R consist of contracts for sale and unexecuted contracts for sale of three of Plaintiffs' properties, including the Wallace property, leaving it to the Court's imagination to ascertain how these contracts demonstrate a loss in value. Wallace's testimony also fails to establish a stigma injury to the property.

Specifically, Wallace claims that she received a verbal offer for her asking price of $500,000.00 from a man named "Amin," whose last name she cannot recall. (Marie Wallace Dep., McKenna Cert., Ex. 0, M.) Wallace claims that he reneged from the agreement after she told him about the release, however, the alleged offeror never gave Wallace the offer in writing and she has no evidence of the offer or "Amin's" motive for withdrawing, aside from her own testimony. Consequently, even construing this evidence in the light most favorable to Plaintiffs, no reasonable jury could find that Plaintiffs' properties were stigmatized on the basis of this evidence alone.

3. Emotional Distress
Defendant also moves for summary judgment of Plaintiffs' claim for emotional distress. Plaintiffs do not respond to this argument in their Opposition, and Defendant is entitled to summary judgment of Plaintiffs' emotional distress claim for Plaintiffs' failure to present evidence of significant distress or physical injury.

A claim for emotional distress cannot succeed absent evidence of physical injury or "severe and substantial" emotional distress, even where a person has a reasonable concern of an enhanced risk of future disease. *Ironbound Health Rights Advisory Com'n v. Diamond Shamrock Chem. Co.,* 243 N.J.Super. 170, 174–75, 578 A.2d 1248 (App.Div.1990) (noting that "[i]n the absence of physical injury, damages are allowed where the resultant emotional distress is severe and substantial" and listing cases). Without some physical injury, mere exposure to toxic chemicals does not give rise to a claim for emotional distress damages. *Id.* (holding plaintiffs unable to sustain emotional distress claim for exposure to chemicals manufactured at plant near their residences); *see also* *Mauro v. Raymark Indus., Inc.,* 116 N.J. 126, 137, 561 A.2d 257 (1989); *Troum v. Newark Beth Israel Med. Ctr.,* 338 N.J.Super. 1, 17, 768 A.2d 177 (App.Div.2001). Because Plaintiffs provided no evidence of significant emotional distress or physical injury, Defendant's motion for summary judgment will be granted.

B. Trespass
Defendant moves for summary judgment of Plaintiffs' claim for trespass. Plaintiffs argue that Defendant's "intentional refusal" to remove the contamination from their property and failure to install remediation equipment amounts to an intentional trespass.[25] (Opp. at 25.)

*12 The Restatement (Second) of Torts defines intentional trespass as:

One who intentionally and without a consensual or other privilege

(a) enters land in possession of another or any part thereof or causes a thing or third person so to do, or

(b) remains thereon, or

(c) permits to remain thereon a thing which the actor or his predecessor in legal interest brought thereon in the manner

stated in §§ 160 and 161, is liable as a trespasser to the other irrespective of whether harm is thereby caused to any of his legally protected interests.

Rest. (2d) Torts § 158.

As Defendant argues, New Jersey has moved away from "such common law claims as trespass and nuisance" in environmental pollution cases. *Mayor and Council of Borough of Rockaway v. Klockner & Klockner,* 811 F.Supp. 1039, 1053 (D.N.J.1993); *Kenney v. Scientific, Inc.,* 204 N.J.Super. 228, 256, 497 A.2d 1310 (1985) ("There is no need for us ... to torture old remedies to fit factual patterns not contemplated when those remedies were fashioned."). Regardless of the continuing viability of trespass claims in the environmental context, however, Plaintiffs have failed to come forward with any evidence supporting their claim and cannot survive summary judgment.

Plaintiffs note that they are "not arguing that Defendants intentionally caused the contamination of their property," but rather are claiming that "defendants have repeatedly refused to perform the horizontal and vertical delineation of the soil and groundwater contamination in the area of the residential properties." (Opp. at 25.) However, no evidence suggests that such measures were necessary to remove contaminants from Plaintiffs' properties. Rather, the record indicates that Defendant consistently complied with NJDEP requirements, including the installation and maintenance of a groundwater recovery system to rehabilitate the aquifer, and the NJDEP never required Defendant to install any sort of remediation equipment on any of the residences. Given that there has been no detection of a gasoline-related contaminant in any Plaintiff's potable well since April 2001, the argument that Defendant permitted contamination to remain on Plaintiffs' properties lacks any viable evidentiary foundation. Defendant's motion for summary judgment of Plaintiffs' trespass claim will be granted.

C. Strict Liability
Plaintiffs originally claimed a cause of action for strict liability under the theory that the handling, storage, or use of gasoline constitutes an abnormally dangerous activity. However, Plaintiffs voluntarily dismissed this claim in their Opposition. (Pl.'s Opp. at 3.) Accordingly, the Court will not address the merits of Plaintiffs' strict liability claim.

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D. Environmental Statutes

1. New Jersey Environmental Rights Act
Plaintiffs allege a right to recover under the New Jersey
Environmental Rights Act ("ERA"), N.J.S.A. 2A:35A–
1 *et seq.* Defendant requests summary judgment on the
grounds that Plaintiffs have not satisfied the ERA's notice
provision, N.J.S.A. 2A:35A–11, and that an ERA claim is not
actionable where the NJDEP has acted to institute and oversee
remediation of the contamination.

 *13 Section 4(a) of the ERA, permits "any person" to
"maintain an action in a court of competent jurisdiction
against any other person to enforce, or to restrain the violation
of, any statute, regulation or ordinance which is designed to
prevent or minimize pollution, impairment or destruction of
the environment." N.J.S.A. 2A:35A–4(a). Although the ERA
itself does not create substantive rights, it confers standing
on private persons to enforce other environmental statutes,
including the New Jersey Spill Compensation and Control
Act ("Spill Act"). ⚑*Rockaway,* 811 F.Supp. at 1054; *Allied
Corp. v. Frola,* 701 F.Supp. 1084, 1091 (D.N.J.1988).

The NJDEP is "entrusted initially with the right to determine
the primary course of action to be taken." *Howell Township
v. Waste Disposal, Inc.,* 207 N.J.Super. 80, 95, 504 A.2d 19
(App.Div.1986) ("In order to be effective, [the NJDEP] must
normally be free to determine what solution will best resolve
a problem on a state or regional basis given its expertise
and ability to view those problems and solutions broadly.").
Consequently, the right of private parties to sue under the
EPA is "an alternative to inaction by the government which
retains primary prosecutorial responsibility." ⚑*Superior Air
Prod. Co. v. NL Indus., Inc.,* 216 N.J.Super. 46, 58, 522 A.2d
1025 (App.Div.1987); ⚑*Rockaway,* 811 F.Supp. at 1054
("[T]he primary goal of the ERA is to limit lawsuits by private
litigants to those instances where the government has not
acted.").

A private ERA suit may be permitted even in the absence
of complete government inaction if the NJDEP has "failed
in its mission ... failed or neglected to act in the best
interest of the citizenry or has arbitrarily, capriciously
or unreasonably acted." *Howell,* 207 N.J.Super. at 96,
504 A.2d 19; ⚑*Morris County Transfer Station, Inc. v.
Frank's Sanitation Serv., Inc.,* 260 N.J.Super. 570, 578,
617 A.2d 291 (App.Div.1992) (permitting private ERA

action where the NJDEP would not address violation for
three years and had taken no enforcement actions against
contaminating defendant who continued operating its illegal
facility two months after receiving a violation notice). Where
NJDEP "action subsequently proves sufficient to protect the
environment," however, NJDEP "action under the Spill Act
is preemptive of private rights under ERA." ⚑*Superior
Air Prod.,* 216 N.J.Super. at 61, 522 A.2d 1025. The
permissibility of private action must be evaluated on a case-
by-case basis. *Id.*

Here the record indicates consistent and pervasive NJDEP
oversight of the remediation process, requiring Defendant
to regularly test Plaintiffs' wells and institute interim and
permanent groundwater recovery systems. Plaintiffs have not
claimed that the NJDEP failed to act or acted unreasonably,
and there are no grounds for finding NJDEP inaction
sufficient to permit a private ERA suit. Furthermore, as
discussed below, Plaintiffs failed to give the NJDEP the
requisite notice of their private suit. Accordingly, Defendant's
motion for summary judgment of Plaintiffs' ERA claim will
be granted.

2. Notice
 *14 Before a private party may commence an action
under the ERA, the party must "at least 30 days prior to
the commencement thereof, direct a written notice of such
intention by certified mail, to the Attorney General, the
Department of Environmental Protection, the governing body
of the municipality in which the alleged conduct has, or
is likely to occur, and to the intended defendant." N.J.S.A.
2A:35A–11. The notice provision is intended to give the
government an adequate opportunity to intervene in the
litigation and to allow the NJDEP:

> to exercise value judgments in
> individual cases, e.g., whether it will
> join in that litigation or enforcement
> proceeding, whether other actions it
> may have taken already with respect
> to the particular problem or offender
> would render the litigation subject
> to collateral estoppel or res judicata
> principles, whether its expertise would
> assist the court, whether broad State
> interests would be sacrificed unduly to

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regional or personal interests by the instigators of that litigation, etc.

Howell, 504 A.2d at 95; *Morris County,* 260 N.J.Super. at 578, 617 A.2d 291 (quoting *Howell* for same).

Because Plaintiffs did not provide the required thirty day notice to the NJDEP or the Attorney General, they are barred from further pursuing their claim under the ERA. Plaintiffs argue that Defendant is judicially estopped from claiming lack of notice for failure to raise this issue at an earlier stage in the case. Plaintiffs analogize the ERA requirement to that of an affidavit of merit, required in certain cases to avoid "unmeritorious and frivolous malpractice lawsuits at an early stage of litigation." *Knorr v. Smeal,* 178 N.J. 169, 197–98, 836 A.2d 794 (2003) (holding judicially estopped defendant's request for summary judgment for plaintiff's failure to file affidavit of merit) (citing *Palanque v. Lambert–Woolley,* 168 N.J. 398, 404, 774 A.2d 501, 505 (2001)); *Ferreira v. Rancocas Orthopedic Assoc.,* 178 N.J. 144, 836 A.2d 779, (2003) (same).

Defendant argues that the ERA notice requirement is more analogous to the notice of intent in the Resource Conservation and Recovery Act (RCRA), which the Supreme Court held to be a jurisdictional prerequisite to suit in *Hallstrom v. Tillamook County,* 493 U.S. 20, 31, 110 S.Ct. 304, 107 L.Ed.2d 237 (1989) ("[C]ompliance with the 60–day notice provision is a mandatory, not optional, condition precedent for suit."); *Public Interest Research Group of N.J., Inc. v. Windall,* 51 F.3d 1179, 1189 (3d Cir.1995) (holding notice provision jurisdictional in context of Clean Water Act ("CWA")); *Hawksbill Sea Turtle v. Federal Emergency Mgmt. Agency,* 126 F.3d 461, 471 (3d Cir.1997) (holding notice provision jurisdictional in context of Endangered Species Act ("ESA")).

However, the language of the notice requirement in RCRA is not entirely analogous to that of the ERA. RCRA states, under the heading of "Actions prohibited" that "No action may be commenced ... prior to 60 days after the plaintiff has given notice of the violation to" the Administrator, the state and the alleged violator. 42 U.S.C.A. § 6972. The ERA lacks the "no action may be commenced" language of the RCRA, CWA, and ESA, and states only that notice must be sent "at least

30 days prior to the commencement" of suit. Consequently, the argument that the plain language of the statute creates a jurisdictional bar is not as strong in the context of the ERA.

*15 Nevertheless, because the purpose of the notice provision is to provide the Attorney General and NJDEP with notice of the suit and opportunity to intervene, *Howell,* 504 A.2d at 95, and not merely to protect defendants, as in the case of the affidavit of merit, Defendant is not judicially estopped from raising Plaintiffs' lack of compliance with the notice provision and is entitled to summary judgment of Plaintiffs' ERA claim.

E. Spill Act Claim

In their complaint, Plaintiffs assert a private right of action under the Spill Act, N.J.S.A. 58:10–23.11 *et seq.* [26] As amended in 1991, the Spill Act authorizes a private cause of action for individuals to recover costs for environmental damage to their property. *Housing Auth. of City of New Brunswick v. Suydam Inv., L.L.C.,* 177 N.J. 2, 18, 826 A.2d 673 (2003). Actions under the Spill Act are limited to clean up and removal costs, *Bahrle v. Exxon Corp.,* 145 N.J. 144, 155, 678 A.2d 225 (1996), defined as:

all direct costs associated with a discharge, and those indirect costs that may be imposed by the department pursuant to section 1 of P.L.2002, c. 37 associated with a discharge, incurred by the State or its political subdivisions or their agents or any person with written approval from the department in the: (1) removal or attempted removal of hazardous substances, or (2) taking of reasonable measures to prevent or mitigate damage to the public health, safety, or welfare, including, but not limited to, public and private property.

N.J.S.A. 58:10–23.11b(d). The Act does not authorize "damages arising from emotional distress, enhanced risk of disease, loss of enjoyment of property, and other economic and financial harm." *Bahrle,* 145 N.J. at 155, 678 A.2d 225. Plaintiffs maintain that the investigation conducted by Ellwood was a reimbursable clean up and removal cost under the Spill Act. As Plaintiffs suggest, because "a discharge cannot be addressed until the contaminants are defined and the extent of the discharge determined," certain forms of investigative costs are implicitly included in the Act. *Metex Corp. v. Federal Ins. Co.,* 290 N.J.Super. 95, 115, 675 A.2d 220 (App.Div.1996).

However, for a private party to obtain reimbursement under the Act, the party must have obtained "written approval from the department," for example, in a memorandum of agreement, prior to incurring the cost. N.J.S.A. 58:10–23.11b(d); *Id.* Such approval permits the NJDEP to "review and approve or disapprove its investigation to date, its proposed remedial action, and its report of the implementation of its action." *Id.; see also* 🚩*Interfaith Cmty Org. v. Honeywell Intern., Inc.,* 263 F.Supp.2d 796, 867 (D.N.J.2003) (concluding "that such costs were approved by and/or incurred at the direction of NJDEP and thus are recoverable

under the Spill Act."). Because Plaintiffs have not obtained NJDEP approval for any cost incurred, including the Ellwood report, Defendant is entitled to summary judgment of Plaintiffs' Spill Act Claim.

*16 The accompanying Order shall enter today.

🚩*Elcock,* 233 F.3d at 741.

All Citations

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Footnotes

1   The following facts are taken from Defendant's statement of undisputed material facts, filed June 24, 2005, ("Undisputed Facts") and Plaintiffs' counterstatement of undisputed facts, filed Oct. 14, 2005, ("Counterstatement Facts"). Plaintiffs did not provide a separate statement of undisputed facts.

Although Plaintiffs dispute the majority of Defendant's statements of fact, Plaintiffs' counterstatements typically provide additional facts without setting forth any conflicting evidence. Where no actual disputes are presented, Defendant's statements will be treated as undisputed. *See e.g.,* 🚩*Tofano v. Reidel,* 61 F.Supp.2d 289, 292 n. 1 (D.N.J.1999) (citing Fed.R.Civ.P. 56(e)) ("This court will ... not consider assertions without evidential support as creating genuine issues of disputed fact."); *Talbot v. United States,* 2005 WL 2917463, *2 (D.N.J.2005) (noting that where the nonmoving party does not submit facts in opposition, "it is entirely appropriate for this court to treat all facts properly supported by the movant to be uncontroverted") (quoting *Allebach v. Sherrer,* No. 04–287, 2005 U.S. Dist. LEXIS 15626, at *5 (D.N.J.2005)).

More generally, Plaintiffs' brief suffers from numerous typographical errors and a dearth of citations to page numbers in the record. This "alone warrants exclusion of the evidence." *See* 🚩*Orr v. Bank of America, NT & SA,* 285 F.3d 764, 774–75 (9th Cir.2002) (holding that party's failure to cite page and line numbers when referencing the deposition merits exclusion of evidence); 🚩*Huey v. UPS, Inc.,* 165 F.3d 1084, 1085 (7th Cir.1999) ("[J]udges need not paw over the files without assistance from the parties."); 🚩*Nissho–Iwai Am. Corp. v. Kline,* 845 F.2d 1300, 1307 (5th Cir.1988) (parties must designate specific facts and their location in the record).

2   Among the original litigants to the suit were also former plaintiffs Michael and Susan Kammerhoff and Norma Simmons. The Kammerhoff plaintiffs were voluntarily dismissed, and plaintiff Norma Simmons died on August 26, 2000.

3   VOCs generally associated with gasoline discharge include MTBE, benzene, toluene, ethylbenzene, xylene (collectively "BTEX"), and tertiary butyl alcohol ("TBA"). The NJDEP has issued a Ground Water Quality Standard ("GWQS") for each of these VOCs, also known as "gasoline-related compounds." MTBE, for example, has a GWQS of 70 parts per billion ("ppb").

4   Although Motiva detected MTBE in thirteen residential wells, not all of these wells are owned by Plaintiffs to this litigation. Of the twenty-seven parcels of property at issue in this suit, only eight of the properties contain wells that ever tested positive for any gasoline-related compound.

5   The direction of water's flow in an aquifer is described as "downgradient," and the direction against the current is "upgradient."

6   In particular, testing revealed emissions in monitoring wells 6–Shallow ("MW–6S") and 7–Deep ("MW–7D"), which lie between the Motiva site and the residential properties. However, the majority of upgradient monitoring wells did not test positive for gasoline-related contaminants. (NJDEP Directive, March 21, 2001 ("March 2001 Directive"), Mairo Cert. in Supp. Def.'s Mot. Summ. J., filed June 24, 2005 ("Mairo Cert."), Ex. O, at 4.)

7   Plaintiffs dispute Defendant's characterization of the CEA, (Counterstatement Facts ¶ 31), on the basis that Defendant proposed the CEA prior to conducting an actual delineation of the plume and that "the Plaintiffs' residential wells could only had [sic] been included in the CEA, if Defendant intended to supply a permanent public water supply to Plaintiffs' properties." While Plaintiffs' contention with the CEA is not entirely clear, Plaintiffs have not provided any evidence indicating that the NJDEP improperly approved the CEA or that the CEA was an inaccurate representation of the boundaries of contaminants in excess of the GWQS.

8   Plaintiffs' properties are: 850 Sicklerville Road; 565, 569, 581, and 583 Berlin–Cross Keys Road; 6, 9, 10, 12, 13, 14, 1, 16, 17, 18, 20 Spring Hollow; 2, 4, 6, and 8 Latham Way; 3, 4, 5, 7, 12, 14, and 15 Donna Marie Court.

9   CW–8 is located approximately 1,000 feet downgradient of the contamination site. (March 2001 Directive at 2.) While active, CW–8 pumps approximately 500 gallons per minute and causes the groundwater to flow southwest. (Ellwood Report at 2.) When CW–8 is not pumping, the groundwater flow is more westerly. (Ellwood Report at 2.)

10   Plaintiff disputes these facts on the basis that:

The Defendant has no data for any portable [sic] water supply of the Plaintiffs prior to July 2000. The Defendant never performed any delineation of the groundwater plume in the areas of the residential properties despite having actual knowledge of such contamination in MW–6, MW–7 and MW–12. See Gallo Certification and Exhibits C, D and E.

(Counterstatement Facts ¶¶ 46–48.) However, because Defendant makes no averment of the presence or absence of contamination prior to July 2000, Defendant's statements are not actually in dispute. Plaintiffs provide no fact indicating an inaccuracy in Defendant's statements regarding the testing of Plaintiffs' wells. Consequently, there is no actual dispute regarding the presence or amount of *detected* gasoline-related compounds.

11   Plaintiffs dispute these statements by citing to Exhibit F of the McKenna certification; however, Exhibit F is the Ellwood report and therefore is not indicative of the NJDEP requirements. Plaintiffs nowhere cite to a statement by the NJDEP requiring Defendant to treat their water or provide them with an alternate water source, and therefore this fact is undisputed.

12   Because this Court will grant Defendant's motion for summary judgment, it will not reach the merits of Defendant's motions to exclude experts Gochfeld, Ellwood, and Gallo.

13   After *Daubert,* Rule 702 was amended to encompass the *Daubert* analysis:

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If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence
or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training,
or education, may testify thereto in the form of an opinion or otherwise, if (1) the testimony is based upon
sufficient facts or data, (2) the testimony is the product of reliable principles and methods, and (3) the
witness has applied the principles and methods reliably to the facts of the case.

Fed.R.Evid. 702. While *Daubert* itself addressed only the admissibility of scientific evidence, the Court

has since noted that courts' gatekeeping obligations extend to all expert testimony. ⚑*Kumho Tire Co. v.
Carmichael,* 526 U.S. 137, 151, 119 S.Ct. 1167, 143 L.Ed.2d 238 (1999).

14   The Court noted that it had "misgivings" about the expert's qualifications in spite of:

> (1) [the expert's] general training in "assessing" individuals, which he received while earning his Ph.D. in
> psychology; (2) his experience, twenty years previous, helping drug addicts reenter the workforce; (3) his
> experience primarily in the last two years dealing with the Virgin Islands Division of Workers' Compensation,
> which he had advised regarding the ability of approximately fifty to sixty-five disabled employees to return to
> their previous jobs; (4) his past experience as an expert witness making lost earning capacity assessments;
> (5) his attendance at two seminars regarding vocational rehabilitation, and his stated familiarity with the
> literature in the area; (6) his membership in two vocational rehabilitation organizations, both of which place
> no restrictions on membership; and (7) the fact that when [the expert] was in school, a degree in vocational
> rehabilitation therapy was not available, but that he received similar training nonetheless.

15   Plaintiffs also argue that "Defendant does not attack the methodology, standard or factual basis for the
opinions," (Opp. at 31), however, it is quite clear from Defendant's motion that the reliability of McDonald's
methodology is hotly disputed.

16   McDonald also appeared unaware of the fact that Plaintiffs' properties are served by potable wells, even
though the potable wells contain the evidence of contamination.

> Q: Do you know whether or not the plaintiffs' properties have potable wells?
>
> A: It's my understanding that they are hooked to a public water system.
>
> Q: If each of the properties did in fact have a potable well, would that be a factor that you were consider
> relevant in your analysis?

Mr. McKenna: You may want to review the documents that you referenced in your report to assist you in
this area. Just separate the Ellwood and Gallo reports. I'm going to go to the men's room.

(Whereupon, a recess is taken.)

Mr. Mairo: I am going to object that Mr. McKenna was basically coaching the witness.

> Back on the record.
>
> A: Your question about whether or not each of these houses were, was, had their own private well on site-
>
> Q: Uh-huh?
>
> A: -it's my understanding that each house is served by wells within and around the neighborhood and
> that Consumer, Consumers Water Company owns those wells and supplies that water to the homes.

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(McDonald Dep. at 36.)

17   McDonald reaches the 35% devaluation figure with the following methodology:

> The subject properties are in the early stages of monitoring, and clean up of the ground water contamination. The properties from Dover Twp. are beyond the clean up stage and into the final stage of recovery, yet they still show a 13% loss in value as compared to similar properties outside of the contaminated area. The subject area is in stage D of recovery, which is the beginning of the remediation process. Based on the acceptance of the Detrimental Condition Model as a viable process for valuing Detrimental Conditions to Real Estate, by the appraisal community and the Subcommittee on Housing and Community Opportunity of the House Committee on Financial Services, it would be logical to assume that the discount to the properties which are the subject of this report, would be 2 to 3 times that of properties in the final stage of recovery. In this case a discount of 35% would be considered reasonable.

(McDonald Report at 31.)

18   Interbay Funding, for example, qualified their statement that they would not lend by noting, "The property would have to be completely cleaned up. They would have to file all necessary documents to the state of NJ and we would require something from the state telling us the property is cleaned up." (McDonald Report at 32.) From this, McDonald concluded that Interbay Funding would not lend on properties such as Plaintiffs', without considering that none of Plaintiffs' properties were contaminated in excess of state standards.

19   In evaluating this data, McDonald states:

> The lenders that did respond have overwhelmingly stated that they would not approve the loan at all, or they would require substantial conditions to the loan. In the case of the subject properties, it can be assumed that a purchaser with private financing or cash would be the only potential buyer of houses in this area.

(McDonald Report at 32.)

20   Because the Court now finds that there is no evidence of any actual injury arising from Defendant's negligence, this Court will not address Defendant's causation argument.

21   Plaintiff argues that Defendant's motion for summary judgment of its negligence claim should be denied on the basis of the doctrine of *res ipsa loquitur.* However, *res ipsa loquitur* acts only to "permit[ ] an inference of defendant's negligence" (i.e., that defendant acted in an unreasonable manner) under particular circumstances. *Jerista v. Murray,* 185 N.J. 175, 192, 883 A.2d 350 (2005). The doctrine does not establish either causation or the presence of damages. *See e.g., Bahrle v. Exxon Corp.,* 279 N.J.Super. 5, 35, 652 A.2d 178 (App.Div.1995) (holding *res ipsa* doctrine inapplicable where "there was a factual dispute as to whether the contamination was a result of plaintiffs' own voluntary acts or neglect"). Accordingly, because Defendant is contesting only causation and damages, the *res ipsa* doctrine does not apply.

22   Gochfeld testifies in his deposition that he created his report without any specific information about the Plaintiffs:

> Q: So, for example, in determining the percentage of the target population that was in high exposure category, that wasn't based on the ground water, your review of the ground water tables that were attached to Mr. Gallo's report?

> A: It was not.

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> Q: That was based purely on just an assumption of yours?
>
> A: It was an assumption based on experience with previous programs or programs that are currently underway in our communities.
>
> Q: Having no specific factual knowledge of the actual exposures in this case?
>
> A: That's correct, these are hypotheticals.

(Gochfeld Dep. at 28–29.)

23   Gochfeld also states that he would not even recommend medical monitoring for the one property with by far the highest detection of MTBE (13.8 ppb at 4 Latham Way) "on this data alone" because "[i]t is possible that a person living there would only be drinking bottled water, would not be in the house very much." (Gochfeld Dep. at 50.)

24   Defendant argues further that New Jersey law does not permit Plaintiffs to recover for stigma damages in the absence of some physical harm to their property. Because Plaintiffs have provided no evidence of any stigma to their property, the Court will not reach Defendant's alternative argument.

25   It is unclear whether Plaintiffs allege negligent trespass since they discuss only the Restatement (Second) of Torts § 158, Intentional Trespass, in their Opposition. Unlike intentional trespass, negligent or reckless trespass requires evidence of "harm to the land, to the possessor, or to a thing or a third person." Rest. Torts 2d § 165; *see also* *Burke v. Briggs,* 239 N.J.Super. 269, 271, 571 A.2d 296 (App.Div.1990) (citing Rest.2d Torts § 158 with approval for another premise); *Karpiak v. Russo,* 450 Pa.Super. 471, 481, 676 A.2d 270 (Pa.Super.1996) (affirming dismissal of trespass claim for entry of dust onto property since the "evidence failed to establish that the dust caused appellants harm"). As discussed previously, Plaintiffs have not provided any evidence of injury to their persons or property. Consequently, to the extent that Plaintiffs are claiming negligent trespass, Defendant is entitled to summary judgment.

26   It is unclear whether Plaintiffs also raise a claim for cleanup and removal costs from the Spill Compensation Fund under N.J.S.A. 58:10–23.11g(a). (Opp. at 12–13.) However, the appropriate procedure to obtain compensation under the Fund is by filing a claim with the administrator of the Fund, "not later than one year after the date of discovery of damage. The administrator shall prescribe appropriate forms and procedures for such claims." N.J.S.A. 58:10–23.11k. In the event "a party, including a potentially responsible party ... contests the amount or validity of" a claim for reimbursement from the Spill Fund, "the dispute is referred to an arbitrator whose decision may be appealed to the Appellate Division," and the arbitrator's decision will be final unless it was "arbitrary, capricious, or unreasonable." *Lacey Municipal Util. Auth. v. New Jersey Dept. of Envir. Prot., Envir. Claims Admin.,* 369 N.J.Super. 261, 273, 848 A.2d 843 (App.Div.2004). Accordingly, this is an improper forum for a Spill Compensation Fund claim.

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Exhibit 26

2013 WL 4675377
Only the Westlaw citation is currently available.
NOT FOR PUBLICATION
United States District Court, D. New Jersey.

Sandra GEISS and Robert Geiss h/w, Plaintiffs,

v.

TARGET CORPORATION and/or Target
Corporation of Minnesota, John Does 1–5
(fictitious persons) and ABC Corps 1–5 (fictitious
corporations), Defendants/Third Party Plaintiff(s),

v.

Virtua Memorial Hospital, Virtua Memorial
Hospital—Mt. Holly, Virtua West, John Does
1–10 (names unknown) and ABC Corps 1–
10 (names unknown), Third Party Defendant(s).

Civil No. 09–2208 (RBK/KMW).
|
Aug. 30, 2013.

Attorneys and Law Firms

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John A. Talvacchia, Stahl & DeLaurentis, P.C., Voorhees, NJ, for Third Party Defendants.

OPINION

KUGLER, District Judge.

 *1 This matter comes before the Court upon the motion of Target Corporation ("Target") for partial summary judgment, pursuant to Federal Rule of Civil Procedure 56, against Sandra and Robert Geiss ("Plaintiffs"). Virtua Memorial Hospital ("Virtua"), a third party defendant in this case, also now moves for summary judgment. For the reasons expressed herein, Target's motion for summary judgment is **DENIED.** However, Virtua's motion for summary judgment is **GRANTED.**

I. FACTS AND PROCEDURAL HISTORY

According to Plaintiffs, this matter arises out of a fall that Plaintiff Sandra Geiss sustained at a Target store in Burlington, New Jersey. Because Plaintiff's medical history and post-fall treatment are relevant to the conflicting theories of causation advanced by both parties, the Court will provide a detailed background to this case. Although the Court presents a composite of facts from Plaintiffs, Target, and Virtua, the Court will construe all facts in the light most favorable to the non-moving parties, as it must at this stage in the litigation.

In January 2006, Plaintiff underwent knee replacement surgery in which her right knee joint was removed and replaced with a prosthetic component. Target's Mot. Summ. J., Ex. P–1 at 1. Plaintiff alleges that on July 25, 2007, she tripped over an uneven rug while passing through the entrance of the Burlington, NJ Target store, landing on her stomach and knees. Id., Ex. B at 2; Target's Statement of Undisputed Material Facts ("SUMF"), ¶ 2. Plaintiff did not experience immediate pain on the day of her fall, but days later developed increasing pain in her right knee and required a cane and walker to ambulate. Id., Ex. T at 33–40. On August 2, 2012, Plaintiff visited her primary care physician, Dr. Chatyrka, complaining of right knee pain. Target's SUMF, ¶ 3. Dr. Chatyrka determined that Plaintiff was suffering from sciatica and recommended that she obtain an X-ray of her right knee. Target's Mot. Summ. J., Ex. C. The X-ray indicated that the prosthetic components were properly positioned and undamaged, but also revealed a fluid collection of unknown origin. Id., Ex. D.

On August 17, 2012, Dr. Schoifet, the orthopedic surgeon who performed Plaintiff's knee replacement in 2006, examined Plaintiff's knee. Id., Ex. E. Dr. Schoifet noted Plaintiff's complaints of increasing knee pain, but found that Plaintiff had no instability in her knee and confirmed that the X-ray demonstrated good positioning of the prosthetic components. Id.; Target's SUMF, ¶ 5–6. He ultimately concluded that Plaintiff suffered a right knee contusion as a result of her fall. Id.

On August 29, 2012, Plaintiff Sandra Geiss presented to Virtual Memorial Hospital complaining of "back pain, leg pain, numbness, pain radiating from back into legs and extreme pain when ambulating." Pls.' Supplemental Statement of Disputed Material Facts ("SDMF"), ¶ 7. A few hours after Plaintiff's arrival, tests revealed that Plaintiff had an elevated white blood cell count, elevated blood pressure, high blood sugar, and a high temperature. Target's Mot. Summ. J. at 4; see also Ex. F at 6–8. Soon thereafter, Plaintiff

was diagnosed with hypoxia and pneumonia. *Id.,* Ex. F at 9. Plaintiff was admitted to the hospital, and then to the Intensive Care Unit, where she was intubated. *Id.,* Ex. I at 2. Blood cultures also revealed that Plaintiff had MSSA (Methicillin–Sensitive Staphylococcus Aureus), a bacterial infection. *Id.* Plaintiff spent some time in the ICU in order to receive treatment for her various ailments and to stabilize her condition. *See* Pls.' Opp'n, Ex. A at 42–43. Dr. Lee does not recall exactly how long Plaintiff remained in the ICU. [1] *Id.* at 42–43.

 *2 Much of the controversy in this case surrounds an "event" which allegedly occurred during Plaintiff's hospitalization. On September 25, 2007, an X-ray of Plaintiff's right knee revealed that her previously intact right knee prosthesis had subluxed (dislocated) by 3cm. Target's Mot. Summ. J., Ex. J. Plaintiff underwent emergency repair surgery on September 26, 2007, while her immune system was still compromised from the treatment of her other ailments. *Id.,* Ex. Q at 99–100. Despite the repair, Plaintiff subsequently developed an infection in her right knee requiring further treatment. Target's SUMF, ¶ 32. The infection persisted, which required doctors to remove the prosthesis and insert an antibiotic spacer. *Id.* at ¶ 33. Ultimately, Dr. Schoifet had to perform a "right knee arthrodesis," or fusion of Plaintiff's right knee. *Id.* at ¶ 34. Plaintiff's knee fusion has caused her significant pain, led to difficulty walking, and altered the range of activities in which she can participate. Dep. of Sandra Geiss at 90–94.

Although the subluxation was discovered on September 25, 2007, Plaintiff has no memory as to when or how it occurred. Target's Mot. Summ. J., Ex. T at 56–57. According to Plaintiff's expert, Dr. Gleimer, this subluxation occurred at some point while Plaintiff was hospitalized, but he cannot pinpoint a specific event, place or date. *Id.,* Ex. P–1 at 3. He does note, however, that the prosthetic is inherently stable and would not sublux on its own. *Id.* This confusion is enhanced due to a number of missing medical records. Specifically, Virtua cannot locate progress notes from August 29, 2007 to September 14, 2007, physician orders from September 4, 2007 to September 17, 2007, medical administration records from September 4, 2007 to September 19, 2007, and flow records from September 14, 2007, September 18, 2007 and October 3, 2007. Target's SUMF, ¶ 35. The Custodian of Records for Virtua, Jennfier Raio, attributes the loss of the records to human error. Virtua's Mot. Summ. J., Ex. D at 64–65.

On the basis of these events, Plaintiffs filed suit against Target on March 26, 2009 in the Superior Court of New Jersey, Burlington County. In the Complaint, Plaintiffs assert claims against Target for negligence and loss of consortium on behalf of Plaintiff Robert Geiss. Target was served on April 6, 2009. Within one month, Target properly moved the matter to this Court. On July 29, 2010, Target impleaded Virtua as a third party defendant in the case. In the Third Party Complaint, Target contends that Plaintiff's knee subluxation constitutes a superseding, intervening cause and that any injuries resulting therefrom are due solely to Virtua's negligence. Target seeks contribution and indemnification from Virtua for any damages for which Target may be liable to Plaintiffs in the underlying suit. Target's Third Party Compl., ¶ 11. Target also claims that it has been prejudiced by Virtua's failure to preserve all of Plaintiff's medical records and asserts a tort action for careless, negligent, and/or intentional spoliation of evidence, seeking contribution and/or indemnification as a remedy. *Id.* at 3–4.

 *3 Both Virtua and Target now move for summary judgment. Target argues that Plaintiff's knee subluxation was neither actually nor proximately caused by Target's negligence. Target also contends that the expert opinion causally relating Plaintiff's fall at Target to her subsequent hospitalization should be barred as a net opinion. In its motion for judgment on the Third–Party Complaint, Virtua argues that neither party has adduced evidence supporting a prima facie case of negligence. Accordingly, Virtua asserts that there is no issue of material fact and that the hospital is entitled to judgment as a matter of law based on the current record.

II. STANDARD OF REVIEW

The court should grant a motion for summary judgment when the moving party "shows that there is no genuine dispute as to any material fact and that the movant is entitled to judgment as a matter of law." Fed.R.Civ.P. 56(a). An issue is "material" to the dispute if it could alter the outcome, and a dispute of a material fact is "genuine" if "a reasonable jury could return a verdict for the non-moving party." *Anderson v. Liberty Lobby, Inc. .,* 477 U.S. 242, 249, 106 S.Ct. 2505, 91 L.Ed.2d 202 (1986); *Matsushida Elec. Indus. Co., Ltd. v. Zenith Radio Corp.,* 475 U.S. 574, 587, 106 S.Ct. 1348, 89 L.Ed.2d 538 (1986) ("Where the record taken as a whole could not lead a rational trier of fact to find for the non-moving party, there is no 'genuine issue for trial.' ") (quoting *First National Bank of Arizona v. Cities Service Co.,* 391

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U.S. 253, 289, 88 S.Ct. 1575, 20 L.Ed.2d 569 (1968)). In deciding whether there is any genuine issue for trial, the court is not to weigh evidence or decide issues of fact. *Anderson,* 477 U.S. at 248. Because fact and credibility determinations are for the jury, the non-moving party's evidence is to be believed and ambiguities construed in her favor. *Id.* at 255; *Matsushida,* 475 U.S. at 587.

Although the movant bears the burden of demonstrating that there is no genuine issue of material fact, the non-movant likewise must present more than mere allegations or denials to successfully oppose summary judgment. *Anderson,* 477 U.S. at 256. The nonmoving party must at least present probative evidence from which jury might return a verdict in his favor. *Id.* at 257. The movant is entitled to summary judgment where the non-moving party fails to "make a showing sufficient to establish the existence of an element essential to that party's case, and on which that party will bear the burden of proof at trial." *Celotex Corp. v. Catrett,* 477 U.S. 317, 322, 106 S.Ct. 2548, 91 L.Ed.2d 265 (1986).

III. DISCUSSION & ANALYIS

Target's Third Party Complaint seeks contribution and indemnification from Virtua for any liability that Target may face in Plantiffs' underlying action. If Target's motion is granted, Virtua's motion for summary judgment would be rendered moot. Therefore, it is prudent for the Court to first address Target's motion for summary judgment.

A. Target's Motion for Partial Summary Judgment

Target moves for summary judgment based on Plaintiffs' alleged failure to establish causation. Target argues that its alleged negligence was neither the actual nor proximate cause of Plaintiff's knee subluxation and the complications resulting therefrom. Target further posits that Plaintiff's knee subluxation was a superseding intervening cause which severs the causal chain of liability. Target also seeks to bar Dr. Gleimer's conclusion that "all hospitalizations subsequent to July 25, 2007 related to Ms. Geiss' knee, back or related infection or problems were caused by the fall at Target." *See* Target's Mot. Summ. J. at 31. Target argues that Dr. Gleimer's statement is a "net opinion," which is unsubstantiated by objective evidence. *Id.* The Court will address these arguments in reverse order, beginning with Target's challenge to Plaintiffs' expert.

a. Sufficiency of Expert Testimony

*4 Target challenges Dr. Gleimer's conclusion that all hospitalizations subsequent to July 25, 2007 are causally related to Plaintiff's fall at Target, arguing that it is a "net opinion" that is unsupported by the factual record. Admissibility of expert testimony is governed by Rule 702, which was amended in 2000 to reflect the Supreme Court decision in *Daubert.* The Rule provides as follows:

> If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise, if (1) the testimony is based upon sufficient facts or data, (2) the testimony is the product of reliable principles and methods, and (3) the witness has applied the principles and methods reliably to the facts of the case.

Fed.R.Evid. 702. This rule requires a court to act as a "gatekeeper" to ensure that expert testimony is both relevant and reliable. *Pineda v. Ford Motor Co.,* 520 F.3d 237, 243 (3d Cir.2008). Rule 702 has a " 'liberal policy of admissibility.' " *Id* . (quoting *Kannankeril v. Terminix Int'l, Inc.,* 128 F.3d 802, 806 (3d Cir.1997)). The burden of showing expert testimony is admissible, once challenged, lies with the offering party. *See Kannankeril,* 128 F.3d at 807.

To be admissible, expert testimony must satisfy three requirements under Rule 702: 1) the witness must be an expert (i.e., must be qualified); 2) the expert must testify about matters requiring scientific, technical, or specialized knowledge (i.e., must be reliable); and 3) the expert's testimony must assist the trier of fact (i.e, must fit). *Id.* at 806 (citing *In re Paoli R.R. Yard PCB Litig. (Paoli II),* 35 F.3d 717, 742 (3d Cir.1994)); *Elcock v. Kmart Corp.,* 233 F.3d 734, 741 (3d Cir.2000) (stating three requirements are qualifications, reliability, and fit). An expert is qualified if

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he " 'possesses specialized expertise.' " *Pineda,* 520 F.3d at 244 (quoting *Schneider ex rel. Estate of Schneider v. Fried,* 320 F.3d 396, 404 (3d Cir.2003)). The qualification requirement is liberally construed. *Id* .

A reliable opinion is "based on the 'methods and procedures of science' rather than on 'subjective belief or unsupported speculation'; the expert must have 'good grounds' for his or her belief." *Paoli II,* 35 F.3d at 742 (quoting *Daubert,* 509 U.S. at 589). The focus of the reliability inquiry is on the expert's principles and methodology, not on his conclusions. *Daubert,* 509 U.S. at 595. In determining reliability, a court may look to several non-exhaustive factors, including:

> (1) whether a method consists of a testable hypothesis; (2) whether the method has been subject to peer review; (3) the known or potential rate of error; (4) the existence and maintenance of standards controlling the technique's operation; (5) whether the method is generally accepted; (6) the relationship of the technique to methods which have been established to be reliable; (7) the qualifications of the expert witness testifying based on the methodology; and (8) the non judicial uses to which the method has been put.

 *5 *Elcock,* 233 F.3d at 745–46 (quoting *Paoli II,* 35 F.3d at 742 n. 8). Finally, an opinion fits a particular case (and thus helps the trier of fact) when there is a " 'connection between the scientific research or test result to be presented and particular disputed factual issues in the case.' " *Oddi v. Ford Motor Co.,* 234 F.3d 136, 145 (3d Cir.2000) (quoting *Paoli II,* 35 F.3d at 743). Fit is an issue of relevance and simply means that scientific validity of the method or principles applies to the issues at hand. *U.S. v. Ford,* 481 F.3d 215, 220 n. 6 (3d Cir.2007).

Target has not raised a proper *Daubert* challenge. Target does not challenge Dr. Gleimer's expertise, the reliability of his methodology, or the relevance of his opinion to this particular case. Target merely challenges the reliability of his conclusions. This is not the "inquiry envisioned by Rule 702."

Daubert v. Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993). As the Supreme Court cautioned, the overarching subject of a challenge under Rule 702 is "the scientific validity and thus the evidentiary relevance and reliability—of the principles that underlie a proposed submission." *Id.* Consequently, the Court's focus "must be solely on principles and methodology, not on the conclusions that they generate." *Id.*

Even construing Target's motion as a challenge to the reliability of Dr. Gleimer's methodology, Target has not raised any justification for barring his opinions. Target first argues that Dr. Gleimer "elected to disregard the medical evidence" and failed to explain how Plaintiff's presentation to the emergency room could have been caused by her fall. Target Mot. Summ. J. at 30. Target also finds significant that Dr. Gleimer cannot state exactly how and when the knee dislocation occurred, but attributes the dislocation to "some event/injury while in the hospital." *Id.* Target further contends that Dr. Gleimer failed to explicitly state that his opinions are based upon a reasonable degree of medical probability or certainty. *Id.* at 31. However, Dr. Gleimer did explain at length his reasons for reaching this conclusion. *See* Dr. Gleimer Dep. at 42–45. The balance of Target's arguments may be properly raised on cross-examination, not on a Rule 702 challenge. Therefore, the Court will deny Target's request to bar Dr. Gleimer's opinions.

b. Negligence

In order to establish negligence under the laws of New Jersey, a plaintiff must establish: (1) a duty of care owed to Plaintiff, (2) a breach of that duty, (3) actual and proximate causation, and (4) damages. *Jersey Cent. Power & Light Co. v. Melcar Utility Co.,* 212 N.J. 576, 594 (2013). Target only challenges Plaintiffs' ability to establish the third prong—actual and proximate causation.

i. Proximate Causation

Target argues that Plaintiff cannot establish that Target's negligence was the proximate cause of her knee subluxation. Target accords little weight to Dr. Gleimer's opinion that an "event" occurred during her hospitalization, but argues that even accepting this conclusion, "it is not foreseeable [that] any treatment for this alleged injury would cause a stable, intact knee prosthetic to dislocate, even if that treatment

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was negligently administered." Target's Mot. Summ. J. at 27. Target also highlights that Plaintiff's own expert "does not state [that] plaintiff was receiving treatment for her back when the knee dislocation occurred, or that the dislocation of plaintiff's was a foreseeable consequence of such treatment." *Id.* However, none of these arguments justify summary judgment.

 *6 Under well-established principles of tort law, "a tortfeasor is generally held answerable for the injuries which result in the ordinary course of events from his negligence and it is generally sufficient if his negligent conduct was a substantial factor in bringing about the injuries." *Rappaport v. Nichols,* 31 N.J. 188, 156 A.2d 1, 9 (N.J.1959). Therefore, to be considered a proximate cause, "conduct need only be a cause which sets off a foreseeable sequence of consequences, unbroken by any superseding cause, and which is a substantial factor in producing the particular injury." *Bendar v. Rosen,* 247 N.J.Super. 219, 588 A.2d 1264, 1269 (N.J.Super.Ct.App.Div.1991) (quoting *Scafidi,* 574 A.2d at 398). The New Jersey Supreme Court has been clear that "[p]roximate cause is a factual issue, to be resolved by the jury after appropriate instruction by the trial court." *Scafidi v. Seiler,* 119 N.J. 93, 574 A.2d 398, 402 (N.J.1990).

Contrary to Target's arguments, Plaintiffs have identified a triable issue of fact as to causation. First, Plaintiffs have offered ample evidence that Plaintiff's visit to the emergency room was spurred by severe back and knee pain. In her deposition, Plaintiff states that she had her husband call an ambulance "because of the excruciating pain she was experiencing in her knee and back." Pls.' SDMF, ¶ 3. Dr. Gleimer opines, and some of the emergency records indicate, that Plaintiff presented to the hospital with complaints of severe left leg pain, back pain, and ambulatory pain. *Id.,* Ex. F; Ex. P–2 at 1. The records also note that Plaintiff complained of "pain to lower back" and that Plaintiff "ambulate[d] slowly without assistance." *Id.* at 6–7.

Although, as Plaintiff concedes, the reasons for her actual admission remain less certain, Plaintiffs have also produced sufficient evidence on this point to survive summary judgment. Plaintiffs' expert, Dr. Gleimer, concluded that there were multiple reasons for Plaintiff's admission and observed that she was treated almost exclusively for her low back pain and sciatica. Pls.' Opp'n at 5(citing Gleimer Dep. at 44–45). Dr. Gleimer notes that these painkillers can also

suppress respiration. *Id.* Dr. Gleimer also highlights that Virtua's Admission Record lists back pain as "one of the conditions chiefly responsible for Ms. Geiss' admission to Virtua." *Id.* (citing Gleimer Dep. at 45–46). Dr. Lee, the admitting doctor on the date in question, also testified that Plaintiff was admitted, at least in part, for "low back pain." *Id.* (citing Lee Dep. at 29). Thus, Plaintiffs have produced adequate evidence for a jury to find that Target's initial negligence was a proximate cause of her knee subluxation.

ii. Actual Causation

Target also argues that Plaintiff's fall was not the actual cause of her knee subluxation. Essentially, Target argues that because an x-ray confirmed that Plaintiff's prosthetic knee was in place after her initial fall and because Dr. Gleimer cannot state with certainty how or when the knee dislocation occurred, Target cannot be the actual cause of her subsequent injury. This argument fundamentally misconstrues the meaning of "actual cause." Actual cause serves as an "important corollary to the proximate cause rule." *See Dawson v. Bunker Hill Plaza Associates,* 289 N.J.Super. 309, 326, 673 A.2d 847 (App.Div.1996). In order to impose liability, a plaintiff must also establish that defendant's negligent conduct was "a substantial factor in bringing about harm to another." *Id.* An actor's conduct is not a substantial factor, "if [the injury] would have been sustained even if the actor had not been negligent.' " *Id.*

 *7 Taking the evidence in the light most favorable to the non-moving party, Plaintiffs have established that Target's conduct was an actual cause of the knee subluxation. Target incorrectly focuses on whether the fall was the direct cause of Plaintiff's injury. However, the law is clear that Target can be liable, "even where there are 'other intervening causes which were foreseeable or were normal incidents of the risk created.' " *Camp v. Jiffy Lube No. 114,* 309 N.J.Super. 305, 309–10, 706 A.2d 1193 (App.Div.1998). Target has not provided any valid basis for summary judgment. Therefore, Target's motion for summary judgment is DENIED.

B. Virtua's Motion for Summary Judgment

Virtua has also moved for summary judgment on Target's Third–Party Complaint against the hospital. Virtua argues that "[n]o party has factually established a prima facie claim against Virtua for negligence." Virtua Mot. Summ. J. at 5. Virtua also contends that to the extent that Target's claim against Virtua alleges medical malpractice, expert

testimony is required to establish a deviation from accepted medical standards. *Id.* at 4, 706 A.2d 1193. Target responds with a number of arguments, none of which are presented with particular lucidity. Target first argues that it need not produce expert testimony because the "common knowledge" exception applies. Target then contends that Virtua's negligent spoliation of evidence entitles Target to an adverse inference. Target also raises the doctrine of "unclean hands" to thwart Virtua's motion for summary judgment. Finally, Target attempts to assert a claim for fraudulent concealment. The Court will address these arguments in turn.

a. Negligence

It is axiomatic that "the mere showing of an incident causing the injury sued upon is not alone sufficient to authorize the finding of an incident of negligence." *Long v. Landy,* 35 N.J. 44, 54, 171 A.2d 1 (1961). As a third-party plaintiff, Target bears the burden of demonstrating the existence of negligence. *See Buckelew v. Grossbard,* 87 N.J. 512, 435 A.2d 1150, 1157 (N.J.1981) ("We start with the basic proposition that ordinarily negligence must be proved and will never be presumed, that indeed there is a presumption against it, and that the burden of proving negligence is on the plaintiff"). Negligence may only be inferred from proven facts and circumstances and cannot be based on speculation or conjecture. *Long,* 35 N.J. at 54, 171 A.2d 1.

Target largely ignores these well-settled principles and attempts to survive summary judgment without providing any competent evidence of Virtua's negligence. Target argues that "the 'event' presumably occurred as a result of the carelessness, negligence, and/or gross negligence of Virtua." Target's Opp'n at 9. However, the law is clear that negligence "will never be presumed." *Buckelew,* 435 A.2d at 1157. Target first attempts to surmount this obstacle by invoking the "common knowledge" exception. According to Target, the common knowledge exception is applicable "where a lay person using ordinary understanding and experience is sufficient to determine a defendant's negligence without the benefit of expert testimony." Target's Opp'n (citing *Bender v. Walgreen Eastern Co. ., Inc.,* 399 N.J.Super. 584, 590, 945 A.2d 120 (N.J.Super.Ct.App.Div.2008)). Target previously raised this same exception in its opposition to Virtua's prior motion to dismiss in relation to the Affidavit of Merit requirement. The Court rejected its application then and will do so again. [2] *See* Doc. No. 30 at 7. Moreover, even if

the Court did apply the common knowledge exception, it would not obviate Target's obligation to establish negligence. It merely alters the proofs upon which a plaintiff may rely to demonstrate a deviation from the standard of care.

*8 In addition to raising the common knowledge exception, Target makes two ill-fated attempts to establish a duty by Virtua. Target Mot. Summ. J. at 11. Target appears to argue that Virtua breached some duty to Target by failing to preserve evidence, which prejudiced Target. However, Target has not identified the source of such a duty. To the extent that Target relies on a common law duty to preserve evidence, Target has not established any of the required elements. The duty to preserve evidence only arises when there is pending or likely litigation between two parties, knowledge of this fact by the alleged spoliator, evidence relevant to the litigation, and the foreseeability that the opposing party would be prejudiced by the disposal of this evidence. *Cockerline v. Menendez,* 411 N.J.Super. 596, 620, 988 A.2d 575 (App.Div.2010). Target also argues that Virtua violated a statutory duty, imposed by N.J. 13:35–6.5, by failing to maintain complete and accurate records. [3] However, the statute does not give rise to a cause of action. *See Proske v. St. Barnabas Med. Ctr.,* 313 N.J.Super. 311, 318–19, 712 A.2d 1207 (App.Div.1998) (finding that N.J.S.A. 26:8–5 does not create a statutory cause of action and that "violation of the statute did not have a causal relation to the physical injury suffered"). Therefore, Target has not alleged any fact, much less provided competent evidence, of Virtua's negligence. [4]

b. Fraudulent Concealment of Evidence

Target also asserts a claim for fraudulent concealment of evidence against Virtua. [5] In order to prove this tort, a plaintiff must demonstrate that: (1) the defendant in the fraudulent concealment action had a legal obligation to disclose evidence in connection with *existing* or *pending* litigation, (2) the evidence was material to the litigation, (3) the plaintiff could not have reasonably obtained the evidence elsewhere, (4) the defendant *intentionally* withheld, altered, or destroyed evidence with purpose to disrupt litigation, (5) Plaintiff was damaged by having to rely on an incomplete record that did not contain the evidence defendant concealed. (emphasis added) *Rosenblit v. Zimmerman,* 166 N.J. 391, 406–07, 766 A.2d 749 (2001). Target has not established these elements. Target has not provided any evidence that the missing records may have been material to this litigation. Target has not even established that Virtua intentionally

withheld the missing entries. Even under the favorable standard of review on summary judgment, Target's claims cannot survive.

In the Third–Party Complaint, Target alleged claims for negligence and what this Court will construe as fraudulent concealment of evidence against Virtua. However, Target has failed to "make a showing sufficient to establish the existence of an element essential to [its] case." ⚑*Celotex,* 477 U.S. at 322. Therefore, the Court will grant Virtua's motion for summary judgment. [6]

IV. CONCLUSION

For the foregoing reasons, Target's Motion for Partial Summary Judgment is DENIED. Virtua's motion for summary judgment is GRANTED. An appropriate order shall issue today.

ORDER

*9 THIS MATTER having come before the Court on the motions of Virtua Memorial Hospital ("Virtua") and Target Corporation ("Target") for summary judgment, pursuant to Federal Rule of Civil Procedure 56, and the Court having considered the moving papers and attached documents, and the responses thereto, and for the reasons expressed in the Opinion issued this date;

IT IS HEREBY ORDERED that Target's motion for summary judgment is **DENIED.**

IT IS HEREBY FURTHER ORDERED that Virtua's motion for summary judgment on Target's Third Party Complaint is **GRANTED.**

All Citations

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Footnotes

1   Plaintiff remained in the hospital until she was discharged on October 11, 2007. Target's SUMF, ¶ 24.

2   In the August 2, 2011 Opinion and Order, the Court stated: "Target has not demonstrated that its claim turns on common knowledge. Target alleges only that 'something' happened while Mrs. Geiss was at Virtua that caused her injuries. Target does not allege that an obvious error by Virtua or its employees caused Mrs. Geiss' injuries. Rather, Target acknowledges that it does not know the exact cause of her injuries. Because Mrs. Geiss received medical treatment, her injuries may have resulted from negligent medical care that requires expert testimony to prove."

3   Virtua notes that Target relies on the wrong statutory provision provision. According to Virtua, NJAC 13:35–6.5 is an administrative code and is not applicable to institutions. Virtua instead posits that NJSA 26:8–5 is the appropriate statutory provision mandating the maintenance of records.

4   Virtua also urges the Court to apply the doctrine of "unclean hands" and deny Virtua's motion for summary judgment. This doctrine "gives expression to the equitable principle that a court should not grant relief to one who is a wrongdoer with respect to the subject matter in the suit." *Faustin v. Lewis,* 85 N.J. 507, 427 A.2d 1105, 1107 (N.J.1981). As with every other argument in Target's opposition, Target has not demonstrated how this doctrine would be applicable. Although it is unfortunate that Virtua could not provide Plaintiff's complete medical record in discovery, Target has not provided any evidence of "wrongdoing with respect to the subject matter in the suit." Jennifer Raio testified that despite their best efforts in searching, her team had not been able to uncover the missing records. Jennifer Raio Dep., Target's Opp'n, Ex. D, 33–34. Moreover, Target has not produced any evidence or testimony linking Virtua's failure to maintain records to Plaintiff's actual injury.

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5   The Third Party Complaint does not explicitly articulate a claim for fraudulent concealment, but it does contain allegations of spoliation of evidence. As Target states, spoliation of evidence claims are recognized as the tort of fraudulent concealment. *See* ⚑ *Rosenblit v. Zimmerman,* 166 N.J. 391, 406, 766 A.2d 749 (2001).

6   Target also seeks an adverse inference jury instruction based on Virtua's alleged spoliation of evidence. Even if the Court were denying Virtua's motion, the Court would not be inclined to address jury instruction requests on a motion for summary judgment.

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Exhibit 28

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organization, or the World Health Organization.

Concise International Chemical Assessment Document 31

N,N-DIMETHYLFORMAMIDE

Please note that the layout and pagination of this pdf file are not identical to those of the printed CICAD

First draft prepared by
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Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organization, and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.

World Health Organization
Geneva, 2001

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

Concise International Chemical Assessment Documents (CICADs) are the latest in a family of publications from the International Programme on Chemical Safety (IPCS) — a cooperative programme of the World Health Organization (WHO), the International Labour Organization (ILO), and the United Nations Environment Programme (UNEP). CICADs join the Environmental Health Criteria documents (EHCs) as authoritative documents on the risk assessment of chemicals.

International Chemical Safety Cards on the relevant chemical(s) are attached at the end of the CICAD, to provide the reader with concise information on the protection of human health and on emergency action. They are produced in a separate peer-reviewed procedure at IPCS. They may be complemented by information from IPCS Poison Information Monographs (PIM), similarly produced separately from the CICAD process.

CICADs are concise documents that provide summaries of the relevant scientific information concerning the potential effects of chemicals upon human health and/or the environment. They are based on selected national or regional evaluation documents or on existing EHCs. Before acceptance for publication as CICADs by IPCS, these documents undergo extensive peer review by internationally selected experts to ensure their completeness, accuracy in the way in which the original data are represented, and the validity of the conclusions drawn.

The primary objective of CICADs is characterization of hazard and dose–response from exposure to a chemical. CICADs are not a summary of all available data on a particular chemical; rather, they include only that information considered critical for characterization of the risk posed by the chemical. The critical studies are, however, presented in sufficient detail to support the conclusions drawn. For additional information, the reader should consult the identified source documents upon which the CICAD has been based.

Risks to human health and the environment will vary considerably depending upon the type and extent of exposure. Responsible authorities are strongly encouraged to characterize risk on the basis of locally measured or predicted exposure scenarios. To assist the reader, examples of exposure estimation and risk characterization are provided in CICADs, whenever possible. These examples cannot be considered as representing all possible exposure situations, but are provided as guidance only. The reader is referred to EHC 170[1] for advice on the derivation of health-based tolerable intakes and guidance values.

While every effort is made to ensure that CICADs represent the current status of knowledge, new information is being developed constantly. Unless otherwise stated, CICADs are based on a search of the scientific literature to the date shown in the executive summary. In the event that a reader becomes aware of new information that would change the conclusions drawn in a CICAD, the reader is requested to contact IPCS to inform it of the new information.

Procedures

The flow chart shows the procedures followed to produce a CICAD. These procedures are designed to take advantage of the expertise that exists around the world — expertise that is required to produce the high-quality evaluations of toxicological, exposure, and other data that are necessary for assessing risks to human health and/or the environment. The IPCS Risk Assessment Steering Group advises the Co-ordinator, IPCS, on the selection of chemicals for an IPCS risk assessment, whether a CICAD or an EHC is produced, and which institution bears the responsibility of the document production, as well as on the type and extent of the international peer review.

The first draft is based on an existing national, regional, or international review. Authors of the first draft are usually, but not necessarily, from the institution that developed the original review. A standard outline has been developed to encourage consistency in form. The first draft undergoes primary review by IPCS and one or more experienced authors of criteria documents in order to ensure that it meets the specified criteria for CICADs.

The draft is then sent to an international peer review by scientists known for their particular expertise and by scientists selected from an international roster compiled by IPCS through recommendations from IPCS national Contact Points and from IPCS Participating Institutions. Adequate time is allowed for the selected experts to undertake a thorough review. Authors are required to take reviewers' comments into account and revise their draft, if necessary. The resulting second draft

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[1] International Programme on Chemical Safety (1994) *Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits.* Geneva, World Health Organization (Environmental Health Criteria 170).

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CICAD PREPARATION FLOW CHART

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1 Taking into account the comments from reviewers.
2 The second draft of documents is submitted to the Final Review Board together with the reviewers' comments.
3 Includes any revisions requested by the Final Review Board.

2

is submitted to a Final Review Board together with the reviewers' comments.

A consultative group may be necessary to advise on specific issues in the risk assessment document.

The CICAD Final Review Board has several important functions:

–   to ensure that each CICAD has been subjected to an appropriate and thorough peer review;
–   to verify that the peer reviewers' comments have been addressed appropriately;
–   to provide guidance to those responsible for the preparation of CICADs on how to resolve any remaining issues if, in the opinion of the Board, the author has not adequately addressed all comments of the reviewers; and
–   to approve CICADs as international assessments.

Board members serve in their personal capacity, not as representatives of any organization, government, or industry. They are selected because of their expertise in human and environmental toxicology or because of their experience in the regulation of chemicals. Boards are chosen according to the range of expertise required for a meeting and the need for balanced geographic representation.

Board members, authors, reviewers, consultants, and advisers who participate in the preparation of a CICAD are required to declare any real or potential conflict of interest in relation to the subjects under discussion at any stage of the process. Representatives of nongovernmental organizations may be invited to observe the proceedings of the Final Review Board. Observers may participate in Board discussions only at the invitation of the Chairperson, and they may not participate in the final decision-making process.

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1. EXECUTIVE SUMMARY

This CICAD on *N*,*N*-dimethylformamide (DMF) was prepared jointly by the Environmental Health Directorate of Health Canada and the Commercial Chemicals Evaluation Branch of Environment Canada based on documentation prepared concurrently as part of the Priority Substances Program under the *Canadian Environmental Protection Act* (CEPA). The objective of assessments on Priority Substances under CEPA is to assess potential effects of indirect exposure in the general environment on human health as well as environmental effects. Occupational exposure was not addressed in this source document. Data identified as of the end of September 1999 (environmental effects) and February 2000 (human health effects) were considered in this review. Information on the nature of the peer review and availability of the source document is presented in Appendix 1. Other reviews that were also consulted include IARC (1999) and BUA (1994). Information on the peer review of this CICAD is presented in Appendix 2. This CICAD was approved as an international assessment at a meeting of the Final Review Board, held in Helsinki, Finland, on 26–29 June 2000. Participants at the Final Review Board meeting are presented in Appendix 3. The International Chemical Safety Card (ICSC 0457) for *N*,*N*-dimethylformamide, produced by the International Programme on Chemical Safety (IPCS, 1999), has also been reproduced in this document.

N,*N*-Dimethylformamide (CAS No. 68-12-2) is an organic solvent produced in large quantities throughout the world. It is used in the chemical industry as a solvent, an intermediate, and an additive. It is a colourless liquid with a faint amine odour. It is completely miscible with water and most organic solvents and has a relatively low vapour pressure.

When emitted into air, most of the DMF released remains in that compartment, where it is degraded by chemical reactions with hydroxyl radicals. Indirect releases of DMF to air, such as transfers from other environmental media, play only a small role in maintaining levels of DMF in the atmosphere. DMF in air is estimated to be photooxidized over a period of days. However, some atmospheric DMF can reach the aquatic and terrestrial environment, presumably during rain events. When DMF is released into water, it degrades there and does not move into other media. When releases are into soil, most of the DMF remains in the soil — presumably in soil pore water — until it is degraded by biological and chemical reaction. Releases to water or soil are expected to be followed by relatively rapid biodegradation (half-life 18–36 h). If DMF reaches groundwater, its anerobic degradation will be slow. The use pattern of DMF is such that exposure of the general population is probably very low.

Since most DMF appears to be released to air in the sample country, and based on the fate of DMF in the ambient environment, biota are expected to be exposed to DMF primarily in air; little exposure to DMF from surface water, soil, or benthic organisms is expected. Based on this, and because of the low toxicity of DMF to a wide range of aquatic and soil organisms, the focus of the environmental risk characterization is terrestrial organisms exposed directly to DMF in ambient air.

DMF is readily absorbed following oral, dermal, or inhalation exposure. Following absorption, DMF is uniformly distributed, metabolized primarily in the liver, and relatively rapidly excreted as metabolites in urine. The major pathway involves the hydroxylation of methyl moieties, resulting in *N*-(hydroxymethyl)-*N*-methylformamide (HMMF), which is the major urinary metabolite in humans and animals. HMMF in turn can decompose to *N*-methylformamide (NMF). In turn, enzymatic *N*-methyl oxidation of NMF can produce *N*-(hydroxymethyl)formamide (HMF), which further degenerates to formamide. An alternative pathway for the metabolism of NMF is oxidation of the formyl group, resulting in *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC), which has been identified as a urinary metabolite in rodents and humans. A reactive intermediate, the structure of which has not yet been determined (possibly methyl isocyanate), is formed in this pathway; while direct supporting experimental evidence was not identified, this intermediate is suggested to be the putatively toxic metabolite. Available data indicate that a greater proportion of DMF may be metabolized by the putatively toxic pathway in humans than in experimental animals. There is metabolic interaction between DMF and alcohol, which, though not well understood, may be due, at least in part, to its inhibitory effect on alcohol dehydrogenase.

Consistent with the results of studies in experimental animals, available data from case reports and cross-sectional studies in occupationally exposed populations indicate that the liver is the target organ for the toxicity of DMF in humans. The profile of effects is consistent with that observed in experimental animals, with gastrointestinal disturbance, alcohol intolerance, increases in serum hepatic enzymes (aspartate aminotransferase, alanine aminotransferase, (-glutamyl transpeptidase, and alkaline phosphatase), and histopathological effects and ultrastructural changes (hepatocellular necrosis, enlarged Kupffer cells, microvesicular steatosis, complex

lysosomes, pleomorphic mitochondria, and fatty changes with occasional lipogranuloma) being observed.

Based on the limited data available, there is no convincing, consistent evidence of increases in tumours at any site associated with exposure to DMF in the occupational environment. Case reports of testicular cancers have not been confirmed in a cohort and case–control study. There have been no consistent increases in tumours at other sites associated with exposure to DMF.

There is also little consistent, convincing evidence of genotoxicity in populations occupationally exposed to DMF, with results of available studies of exposed workers (to DMF and other compounds) being mixed. The pattern of observations is not consistent with variations in exposure across studies. However, in view of the positive dose–response relationship observed in the one study in which it was investigated, this area may be worthy of additional work, although available data on genotoxicity in experimental systems are overwhelmingly negative.

DMF has low acute toxicity and is slightly to moderately irritating to the eyes and skin. No data were identified regarding the sensitization potential of DMF. In acute and repeated-dose toxicity studies, DMF has been consistently hepatotoxic, inducing effects on the liver at lowest concentrations or doses. The profile of effects includes alterations in hepatic enzymes characteristic of toxicity, increases in liver weight, progressive degenerative histopathological changes and eventually cell death, and increases in serum hepatic enzymes. A dose–response has been observed for these effects in rats and mice following inhalation and oral exposure. Species variation in sensitivity to these effects has been observed, with the order of sensitivity being mice > rats > monkeys.

Although the database for carcinogenicity is limited to two adequately conducted bioassays in rats and mice, there have been no increases in the incidence of tumours following chronic inhalation exposure to DMF. The weight of evidence for genotoxicity is overwhelmingly negative, based on extensive investigation in *in vitro* assays, particularly for gene mutation, and a more limited database *in vivo*.

In studies with laboratory animals, DMF has induced adverse reproductive effects only at concentrations greater than those associated with adverse effects on the liver, following both inhalation and oral exposure. Similarly, in well conducted and reported primarily recent developmental studies, fetotoxic and teratogenic effects

have been consistently observed only at maternally toxic concentrations or doses.

Available data are inadequate as a basis for assessment of the neurological or immunological effects of DMF.

The focus of this CICAD and the sample risk characterization is primarily effects of indirect exposure in the general environment.

Air in the vicinity of point sources appears to be the greatest potential source of exposure of the general population to DMF. Based on the results of epidemiological studies of exposed workers and supporting data from a relatively extensive database of investigations in experimental animals, the liver is the critical target organ for the toxicity of DMF. A tolerable concentration of 0.03 ppm (0.1 mg/m^3) has been derived on the basis of increases in serum hepatic enzymes.

Data on the toxicity of DMF to terrestrial vascular plants have not been identified. Effect concentrations for indicators of the potential sensitivities of trees, shrubs, and other plants are high; hence, it is unlikely that terrestrial plants are particularly sensitive to DMF. For other terrestrial organisms, an estimated no-effects value of 15 mg/m^3 has been derived based on a critical toxicity value for hepatic toxicity in mice divided by an application factor. Comparison of this value with a conservative estimated exposure value indicates that it is unlikely that DMF causes adverse effects on terrestrial organisms in the sample country.

2. IDENTITY AND PHYSICAL/CHEMICAL PROPERTIES

N,N-Dimethylformamide (CAS No. 68-12-2) is a colourless liquid at room temperature with a faint amine odour (BUA, 1994). There are many synonyms for this compound, the most common being the acronym DMF. The molecular mass of DMF is 73.09, as calculated from its empirical formula (C_3H_7NO). DMF sold commercially contains trace amounts of methanol, water, formic acid, and dimethylamine (BUA, 1994).

DMF is miscible in all proportions with water and most organic solvents (Syracuse Research Corporation, 1988; Gescher, 1990; BUA, 1994; SRI International, 1994). DMF is also a powerful solvent for a variety of organic, inorganic, and resin products (SRI International, 1994). At temperatures below 100 °C, DMF

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Table 1: Physical and chemical properties of DMF.

Property	Value	Reference	Values used in fugacity calculations[a]
Molecular mass	73.09		73.09
Vapour pressure (Pa at 25 °C)	490	Riddick et al. (1986)	490
Solubility (g/m³)	miscible	BUA (1994)	1.04×10^6
Log K_{ow}	! 1.01	Hansch et al. (1995)	! 1.01
Henry's law constant (Pa·m³/mol at 25 °C)	0.0345 0.0075	Bobra[b] BUA (1994)	0.034 53[c]
Density/specific gravity (g/ml at 25 °C)	0.9445	WHO (1991)	
Melting point (°C)	! 60.5	WHO (1991)	! 60.5 °C
Boiling point (°C)	153.5	WHO (1991)	
Half-life in air (h)	approx. 192	estimated from propane	170
Half-life in water (h)	18 36	Dojlido (1979) Ursin (1985)	55
Half-life in soil (h)	assumed to be equivalent to that in water		55
Half-life in sediment (h)	–		170
Half-life in suspended sediment (h)	–		55
Half-life in fish (h)	–		55
Half-life in aerosol (h)	–		5
Odour threshold	0.12–60 mg/m³	WHO (1991)	

[a]   Discussed in section 11.1.3, Sample risk characterization.

[b]   Collection of notes and modelling results submitted by A. Bobra, AMBEC Environmental Consultant, to Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, 1999.

[c]   Based upon vapour liquid equilibrium data (Hala et al., 1968), as calculated in DMER & AEL (1996).

remains stable in relation to light and oxygen (BUA, 1994). Temperatures in excess of 350 °C are required for DMF to decompose into carbon monoxide and dimethylamine (Farhi et al., 1968).[1]

Some important physical and chemical properties of DMF are summarized in Table 1. A vapour pressure of 490 Pa was recommended by Riddick et al. (1986). Because DMF is a miscible compound, it is preferable to determine the Henry's law constant experimentally. However, no experimental data were identified in the literature, and the calculated Henry's law constant of DMF remains uncertain (DMER & AEL, 1996).[2] The

[1] Also notes from N.J. Bunce, University of Guelph, Guelph, Ontario, to A. Chevrier, Environment Canada, 1 June 1998.

[2] Also collection of notes and modelling results submitted by A. Bobra, AMBEC Environmental Consultant, to Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, 1999.

octanol/water partition coefficient (K_{ow}) was determined by a shake flask experiment (Hansch et al., 1995).

The conversion factor for DMF in air is as follows (WHO, 1991): 1 ppm = 3 mg/m^3.

3. ANALYTICAL METHODS

The following information on analytical methods for the determination of DMF in workplace air and biological media has been derived from WHO (1991) and Environment Canada (1999a).

3.1   DMF in workplace air

Colorimetric methods (based on the development of a red colour after the addition of hydroxylamine chloride as alkaline solution) that have often been utilized in the past are not specific (Farhi et al., 1968). Methods of choice more recently are high-performance liquid chromatography (HPLC) or gas chromatography – mass spectrometry (GCMS). Lauwerys et al. (1980)

described a simple spectrophotometric method for measuring DMF vapour concentrations. Gas–liquid chromatography (GLC) is now the method of choice (Kimmerle & Eben, 1975a; NIOSH, 1977; Muravieva & Anvaer, 1979; Brugnone et al., 1980; Muravieva, 1983; Stransky, 1986). Detector tubes, certified by the US National Institute for Occupational Safety and Health, or other direct-reading devices calibrated to measure DMF (Krivanek et al., 1978; NIOSH, 1978) can be used. HPLC analysis (Lipski, 1982) can also be used. Mass spectrometric analysis for DMF in expired air has been described by Wilson & Ottley (1981), with a lower limit of detection of 0.5 mg/m^3. Figge et al. (1987) reported determination in air involving the enrichment of an organic polymer, thermal desorption of the adsorbed species, and qualitative determination by GCMS. The lower limit of detection was 5 ng/m^3. A NIOSH (1994) gas chromatographic (GC) method has an estimated detection limit of 0.05 mg per sample.

3.2   DMF and metabolites in biological media

DMF is extensively absorbed through the skin, its metabolism and kinetics are well known, and urinary metabolites exist that can be accurately measured. As a result, biological monitoring has been extensively used in the assessment of the absorbed amounts in occupationally exposed populations. The metabolite most often analysed is *N*-methylformamide (NMF), and several GC methods exist (Ikeda, 1996). Using nitrogen-sensitive detection, the limit of detection is 0.1 mg/litre.

4. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

4.1   Natural sources

BUA (1994) identified no known natural sources of DMF. However, DMF is a possible product of the photochemical degradation of dimethylamine and trimethylamine (Pellizzari, 1977; Pitts et al., 1978; US EPA, 1986). Both are commonly occurring natural substances and are also used in industrial applications (European Chemicals Bureau, 1996a, 1996b).

4.2   Anthropogenic sources

Identified data on releases are restricted to the country of origin of the source document (Canada). They are presented here in the context of an example of an emissions profile.

In 1996, just over 16 tonnes of DMF were released from various industrial locations in Canada, of which 93% (15 079 kg) were emitted to the atmosphere and the remainder to water (245 kg), wastewater (204 kg), landfill sites (26 kg), or deep-well injection (669 kg) (Environment Canada, 1998). The Canadian market for DMF is quite small, with an estimated domestic consumption in the range of less than 1000 tonnes/year (SRI International, 1994; Environment Canada, 1998). The petrochemical sector was responsible for 84% (12.7 tonnes) of the reported atmospheric releases. Releases from the pharmaceutical industry accounted for 87% (0.212 tonnes) of total releases to water. Total release volumes from Canadian industrial sectors include 13.3 tonnes from the petrochemical sector, 1.2 tonnes from manufacture of pharmaceuticals, 0.7 tonnes from dye and pigment manufacture, 0.6 tonnes from polyvinyl chloride coating operations, 0.1 tonnes from its use as a solvent in pesticide manufacture, 0.07 tonnes from paint/finisher and paint remover manufacture, and 0.09 tonnes from other miscellaneous industrial sectors. For 1996, a reported total quantity of 0.056 tonnes was released (0.023 tonnes to air, 0.033 tonnes to water) by the producer during chemical synthesis of DMF (Environment Canada, 1998). Less than 1 tonne of DMF was released from wastewater treatment facilities and in landfills (Environment Canada, 1998). With a few exceptions, most industries reported little to no seasonal variation in releases (Environment Canada, 1998).

In the USA, between 23 and 47 million kilograms of DMF were produced in 1990 (US EPA, 1997).

World production of DMF is estimated to be 125 000 tonnes (Marsella, 1994).

The total consumption of DMF in Western Europe in 1989 was reported to be 55 000 tonnes (BUA, 1994). The production capacity was estimated to be 60 000 and 19 000 tonnes in the former Federal Republic of Germany and German Democratic Republic, respectively, 16 000 tonnes in Belgium, 15 000 tonnes in England, and 5000 tonnes in Spain (BUA, 1994).

Although small accidental releases (e.g., leakage of a storage tank or spill from a barrel) may remain unreported, available information suggests that spills of DMF during use, storage, or transport are not a significant route of entry to the environment (Environment Canada, 1999a).

The quantity of DMF in landfill sites should be small. The total quantity of DMF used in formulation of products (other than pesticides) appears to be small in comparison to its use as a manufacturing aid, cleaner, or degreaser (Environment Canada, 1998). As such,

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consumer products deposited in landfill sites should contain little or no DMF. The industrial DMF deposited directly in landfill sites consists only of residues remaining after incineration (Environment Canada, 1998).

4.3   Uses

DMF is used commercially as a solvent in vinyl resins, adhesives, pesticide formulations, and epoxy formulations; for purification and/or separation of acetylene, 1,3-butadiene, acid gases, and aliphatic hydrocarbons; and in the production of polyacrylic or cellulose triacetate fibres and pharmaceuticals (WHO, 1991; IARC, 1999). DMF is also used in the production of polyurethane resin for synthetic leather (Fiorito et al., 1997).

5. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION

5.1   Air

The atmospheric pathway is particularly important in determining exposure to DMF. This is due to the fact that industrial releases of DMF into air appear to be considerably larger than releases to other environmental media (BUA, 1994; Environment Canada, 1998).

Because of the complete miscibility of DMF in water, atmospheric DMF may be transported from air into surface water or soil pore water during rain events (DMER & AEL, 1996).[1] Atmospheric DMF should be present in the vapour phase and therefore should be readily available for leaching out by rainfall (US EPA, 1986).[2] Although the efficiency and rate of washout are unknown, precipitation events (i.e., rain, snow, fog) likely shorten the residence time of DMF in the atmosphere. As water has an atmospheric half-life of approximately 4 days at Canadian latitudes, this can be considered the minimum atmospheric half-life of DMF in relation to precipitation.[1]

Chemical degradation of DMF in air is likely due to reaction with hydroxyl radicals (Hayon et al., 1970). The

possibility of photochemical decomposition (i.e., direct photolysis) of DMF is extremely small (Grasselli, 1973; Scott, 1998). Other chemical degradation processes — for example, reaction with nitrate radicals — are not known to significantly affect the fate of DMF in air.

The reaction rate constant (k_{OH}) for the formamide functional group is unknown. However, the degradation half-life of DMF can be roughly estimated by comparing DMF with other compounds in terms of their relative atmospheric reactivity.

Based on experiments in chambers, reactivity for DMF relative to propane is low (Sickles et al., 1980). The k_{OH} of propane is 1.2×10^{-12} cm^3/molecule per second (Finlayson-Pitts & Pitts, 1986). Using the global average hydroxyl radical concentration of 7.7×10^5 molecules/cm^3 (Prinn et al., 1987) and the calculation method proposed by Atkinson (1988), the half-life of propane is estimated at approximately 8 days.

Although the degradation half-life of DMF in air cannot be estimated with certainty, the available evidence therefore suggests that the half-life is at least 8 days (192 h). The mean half-life used for fugacity-based fate modelling was 170 h, as it is frequently used to represent a half-life range of 100–300 h (DMER & AEL, 1996). This half-life may be underestimated; however, sensitivity analysis on the fugacity-based results indicates that percent partitioning estimates are not sensitive to this parameter, but estimated concentrations are affected.[3]

5.2   Surface water and sediment

Once released into surface water, DMF is unlikely to transfer to sediments, biota, or the atmosphere. With a K_{ow} of ! 1.01 (Hansch et al., 1995), DMF remains in the dissolved form and is not expected to adsorb to the organic fraction of sediments or suspended organic matter. This K_{ow} also suggests that DMF does not concentrate in aquatic organisms (BUA, 1994); indeed, no bioaccumulation was observed in carp during an 8-week bioaccumulation test (Sasaki, 1978). With a Henry's law constant of 0.0345 Pa·m^3/mol, volatilization from water is expected to be slight (BUA, 1994).[3]

The overall rate of chemical degradation is expected to be very slow in surface water.

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[1] Also letter from D.R. Hastie, York University, Toronto, Ontario, to P. Doyle, Environment Canada, 1998.

[2] Also technical note from N.J. Bunce, University of Guelph, Guelph, Ontario, to B. Scott, Environment Canada, dated 10 February 1998.

[3] Collection of notes and modelling results submitted by A. Bobra, AMBEC Environmental Consultant, to Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, 1999.

Photochemical decomposition is unlikely in water (Grasselli, 1973; US EPA, 1986). The photooxidation half-life of DMF in water was estimated experimentally at 50 days and would be even longer in the natural environment where other compounds compete for reaction with hydroxyl radicals (Hayon et al., 1970). The rate of hydrolysis of amides like DMF at normal temperatures in laboratory studies is extremely slow, even under strong acid or base conditions (Fersht & Requena, 1971; Eberling, 1980). The low temperature (generally less than 20 °C) and near-neutral pH of natural surface water therefore limit and almost preclude the hydrolysis of DMF under normal environmental conditions (Frost & Pearson, 1962; Langlois & Broche, 1964; Scott, 1998).

Biodegradation appears to be the primary degradation process in surface water. Under experimental conditions, DMF was degraded, either aerobically or anaerobically, by various microorganisms and algae in activated sludges, over a wide range of concentrations (Hamm, 1972; Begert, 1974; Dojlido, 1979). Intermediate biodegradation products include formic acid and dimethylamine, which further degrade to ammonia, carbon dioxide, and water (Dojlido, 1979; Scott, 1998). In some studies, acclimation periods of up to 16 days preceded quantitative degradation (Chudoba et al., 1969; Gubser, 1969). Extended adaptation under specific experimental conditions may also account for negative degradation results observed in a few studies with incubation times #14 days (Kawasaki, 1980; CITI, 1992). Limited degradation was reported in seawater (range 1–42%) (Ursin, 1985), and no degradation was found after 8 weeks' incubation under anaerobic conditions (Shelton & Tiedje, 1981).

Biodegradation of DMF in receiving surface waters is unlikely to be affected by the inherent toxicity of DMF and its biodegradation products. Concentrations above 500 mg/litre in effluent reduced the efficiency of treatment systems using activated sludge (Thonke & Dittmann, 1966; Nakajima, 1970; Hamm, 1972; Begert, 1974; Carter & Young, 1983). However, even with continuous releases, such high concentrations of DMF are not anticipated in natural waters.

In a river die-away test, an initial concentration of 30 mg DMF/litre completely disappeared within 3 and 6 days from unacclimated and acclimated water, respectively (Dojlido, 1979). The mineralization rate of DMF in seawater was less than 3% in 24 h for initial concentrations of 10 µg/litre and 100 µg/litre. However, 20% was mineralized in 24 h at a concentration of 0.1 µg/litre (Ursin, 1985). A half-life of 55 h was used for water in the fugacity-based fate modelling described in

section 5.4 (DMER & AEL, 1996).[1,2] No information is available on the half-life of DMF in sediments. DMER & AEL (1996) recommend a half-life in sediment of 170 h based on the assumption that reactivity in sediment is slower than in soil.

5.3   Soil and groundwater

Fugacity-based fate modelling and the miscibility of DMF indicate that some of the DMF released into the atmosphere can reach the ground, in part, at least, through rainfall (DMER & AEL, 1996).[1,2] Once in soils, DMF will be degraded by chemical and biological processes or leached into groundwater.

As rain fills the available pore space in soils, DMF is incorporated into the pore water. With an octanol/water partition coefficient of !1.01 (Hansch et al., 1995), DMF will not tend to adsorb to humic material. Weak bonds with the mineral phase are possible but likely insignificant because of the high solubility of DMF.[3]

Biological degradation and, to a lesser extent, chemical processes operating in surface water would also likely affect DMF contained in soil pore water (Scott, 1998). As for surface water, biodegradation should therefore be the primary breakdown mechanism in soils. A soil bacterial culture acclimated to small amounts of petroleum and petroleum products degraded DMF under aerobic conditions within 18 h (Romadina, 1975), indicating a soil biodegradation half-life similar to the one observed in water. A somewhat longer conservative half-life of 55 h was used in fugacity-based fate modelling (DMER & AEL, 1996).[1,2]

The miscibility of DMF and its low Henry's law constant indicate limited volatilization from moist soils (BUA, 1994). However, DMF will be efficiently removed from soils by leaching into groundwater, likely at the same speed as water percolates through the soil.[4] This is

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[1] Also technical note sent from R. Beauchamp, Health Canada, to A. Chevrier, Environment Canada, 1998.

[2] Also collection of notes and modelling results submitted by A. Bobra, AMBEC Environmental Consultant, to Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, 1999.

[3] Letter from K. Bolton, University of Toronto, Toronto, Ontario, to A. Chevrier, Environment Canada, dated 8 June 1998.

[4] Technical note from S. Lesage to B. Elliott, Environment Canada, dated 26 November 1997.

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supported by a calculated organic carbon/water partition coefficient (K_{oc}) of 7 (Howard, 1993) and a soil sorption coefficient (K_{om}) of about 50, estimated from quantitative structure–activity relationships (Sabljic, 1984; US EPA, 1986), which both indicate that DMF is mobile in soils. If it reaches groundwater, DMF will be slowly degraded anaerobically (Scott, 1998).[1]

5.4   Environmental distribution

Fugacity modelling was conducted to provide an overview of key reaction, intercompartment, and advection (movement out of a system) pathways for DMF and its overall distribution in the environment. A steady-state, non-equilibrium model (Level III fugacity modelling) was run using the methods developed by Mackay (1991) and Mackay & Paterson (1991). Assumptions, input parameters, and results are summarized in Environment Canada (1999a) and presented in detail in DMER & AEL (1996) and by Beauchamp[2] and Bobra.[3] Modelling predictions do not reflect actual expected concentrations in the environment but rather indicate the broad characteristics of the fate of the substance in the environment and its general distribution among the media.

Modelling results identify air as an important exposure medium. If DMF is emitted into air, fugacity modelling predicts that 61% of the chemical will be present in air, 32% in soil, and only 7% in water. These results suggest that most of the DMF released into air will remain in that compartment, where it will be degraded by chemical reactions. They also indicate that some atmospheric DMF can reach the aquatic and terrestrial environment — presumably in rain and runoff (Scott, 1998).[4] However, the quantity of DMF available for entrainment in rain and runoff is limited by degradation in the atmosphere.

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[1] Technical note from S. Lesage to B. Elliott, Environment Canada, dated 26 November 1997.

[2] Technical note sent from R. Beauchamp, Health Canada, to A. Chevrier, Environment Canada, 1998.

[3] Collection of notes and modelling results submitted by A. Bobra, AMBEC Environmental Consultant, to Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, 1999.

[4] Also letter from S. Lei, Atomic Energy Control Board of Canada, to A. Chevrier, Environment Canada, dated 11 June 1998.

Fugacity modelling also indicates that when DMF is continuously discharged into either water or soil, most of it can be expected to be present in the receiving medium. For example, if it is released into water, 99% of the DMF is likely to be present in the water, and subsequent transport into sediment or bioconcentration in biota is not likely to be significant. When releases are into soil, 94% of the material remains in the soil — presumably in soil pore water (Scott, 1998). Therefore, indirect releases of DMF to air, such as transfers from other environmental media, play only a small role in maintaining levels of DMF in the atmosphere.

It is important to note that fugacity-based partitioning estimates are significantly influenced by input parameters such as the Henry's law constant, which, in this case, is highly uncertain. Therefore, the above partitioning estimates are also uncertain.

6. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

6.1   Environmental levels

6.1.1   Ambient air

Concentrations of DMF in stack emissions of two Canadian industries were less than 7.5 mg/m^3 (Environment Canada, 1998, 1999b). Data on concentrations in ambient air around these sources are not available.

In Lowell, Massachusetts, USA (Amster et al., 1983), DMF was detected in the air over an abandoned chemical waste reclamation plant (0.007 mg/m^3), a neighbouring industry (>0.15 mg/m^3), and a residential area (0.024 mg/m^3). Ambient air samples collected in the northeastern USA in 1983 ranged from less than 0.000 02 to 0.0138 mg DMF/m^3 (Kelly et al., 1993, 1994). In samples taken in 1983, levels of DMF were generally less than 0.02 mg/m^3 at a hazardous waste site in unsettled wind conditions, possibly as high as 9 mg/m^3 at nearby industrial sites, and less than 0.02 mg/m^3 in adjoining residential areas (Clay & Spittler, 1983).

A range of 0.000 11 – 0.0011 mg/m^3 was reported in Japan in 1991, but specific locations and proximity to sources were not provided (Environment Agency Japan, 1996). In Germany, a concentration of $0.005 µg DMF/m^3 was detected in air (Figge et al., 1987).

6.1.2   Surface water and sediment

DMF was detected (detection limit 0.002 mg/litre) in only 1 of 204 surface water samples collected between August 1975 and September 1976 from 14 heavily industrialized river basins in the USA (Ewing et al., 1977). The Environment Agency Japan (1996) reported concentrations between 0.0001 and 0.0066 mg/litre in 18 out of 48 water samples taken in 1991. In addition, in 24 water samples collected in 1978, levels were below the detection limits of 0.01–0.05 mg/litre (Environment Agency Japan, 1985). The proximity of these measurements to industrial sources is not known.

In Canada, monitoring data are available for effluents at one southern Ontario location, which released less than ~0.03 tonnes into surface water in 1996 (Environment Canada, 1998). The facility reported a range of <1–10 mg DMF/litre in effluents, but has since established a wastewater treatment plant, which reduced its effluent concentrations to non-detectable levels (detection limit 0.5 mg/litre). DMF was detected in 1 of 63 industrial effluents in the USA at a detection limit of approximately 0.01 mg/litre (Perry et al., 1979). The US Environmental Protection Agency (EPA)[1] also cited an effluent concentration of 0.005 mg/litre at a sewage treatment plant in 1975.

The properties of DMF and fugacity modelling indicate negligible accumulation of DMF in sediments (BUA, 1994; Hansch et al., 1995; DMER & AEL, 1996).[2,3] However, concentrations of 0.03–0.11 mg/kg were reported in sediments (9 out of 48 samples) in Japan (Environment Agency Japan, 1996). No information was provided on proximity to sources of DMF, sediment characteristics, or hydrological regimes. In addition, because information on sampling and analytical methods was not provided, the quality of these data cannot be assessed. In 24 sediment samples collected in 1978 at unspecified locations in Japan, levels were below the detection limits of 0.1–0.3 mg/kg (Environment Agency Japan, 1985).

[1] Group STORET search on DMF, obtained from J. Boyd, US EPA (storet@epamail.eap.gov), on 30 July 1999.

[2] Also technical note sent from R. Beauchamp, Health Canada, to A. Chevrier, Environment Canada, 1998.

[3] Also collection of notes and modelling results submitted by A. Bobra, AMBEC Environmental Consultant, to Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, 1999.

6.1.3   Soil and groundwater

In 3 of 23 groundwater samples collected in the USA, concentrations ranged from 0.05 to 0.2 mg/litre, with an average value of 0.117 mg/litre (Syracuse Research Corporation, 1988).[1]

6.2   Human exposure

6.2.1   Drinking-water

Although DMF was listed as a contaminant in a survey of drinking-water in the USA, quantitative data were not reported (Howard, 1993).

6.2.2   Food

Data on concentrations of DMF in foods were not identified.

6.2.3   Multimedia study

A Health Canada-sponsored multimedia exposure study for DMF and other volatile organic compounds was conducted in 50 homes in the Greater Toronto Area in Ontario, Nova Scotia, and Alberta (Conor Pacific Environmental, 1998). DMF was not detected in indoor air samples from the 50 residences (detection limit 3.4 µg/m^3). It was also not detected in tap water samples, although the limit of detection was high (0.34 µg/ml). DMF was not recovered reproducibly in composite food or beverage samples in this study.

6.2.4   Exposure of the general population

Identified data on concentrations of DMF in environmental media in Canada were insufficient to allow estimates of population exposure to be developed; for water, either quantitative data on concentrations are unreliable[4] or DMF has not been detected, using analytical methodology with poor sensitivity (Conor Pacific Environmental, 1998).

Non-pesticidal use of DMF in Canada is small and restricted primarily to industrial applications. Most DMF released into the environment in Canada during such use is emitted to air. Most DMF remains in the medium of release prior to degradation. Therefore, the greatest potential for exposure of the general population to DMF

[4] Technical notes regarding data from Environmental Monitoring and Reporting Branch, Ontario Ministry of Environment and Energy, sent to J. Sealy, Health Canada, 1996.

from non-pesticidal sources is in air in the vicinity of industrial point sources.

Based upon dispersion modelling of releases in Canada from the highest emitter over a 1-km radius, 100 m in height, the estimated ambient concentration is 110 µg/m^3. Although this value is comparable to levels measured under similar conditions in other countries, it is based on very conservative assumptions; taking into account more likely conditions, including some loss due to advection, estimated concentrations would be 10- to 100-fold less (i.e., 11 or 1.1 µg/m^3).

Based on lack of detection in a multimedia study, levels of DMF in indoor air of 50 homes in Canada were less than 3.4 µg/m^3 (Conor Pacific Environmental, 1998).

6.2.5 *Occupational exposure*

Occupational exposure to DMF may occur in the production of the chemical itself, other organic chemicals, resins, fibres, coatings, inks, and adhesives (IARC, 1999). Exposure may also occur during use of these coatings, inks, and adhesives in the synthetic leather industry, in the tanning industry, and as a solvent in the repair of aircraft (Ducatman et al., 1986; IARC, 1989).

Based on data from the National Exposure Data Base, maintained by the United Kingdom Health and Safety Executive, concentrations of DMF in workplace air in the manufacture of textiles ranged from 0.1 to 10.5 ppm (0.3 to 7.5 mg/m^3) in 16 facilities.[1] For the six facilities where data were reported, the 8-h time-weighted average (TWA) concentration ranged from 4 to 12.4 ppm (12 to 37.2 mg/m^3). At six facilities where plastic was manufactured, concentrations ranged from 0.1 to 0.7 ppm (0.3 to 2.1 mg/m^3). At 11 facilities for plastics processing, the range of concentrations was from 4 to 44 ppm (12 to 132 mg/m^3); the range of 8-h threshold limit values (TLVs) at six of the facilities was 5–38 ppm (15–114 mg/m^3).

In the USA between 1981 and 1983, approximately 125 000 workers were potentially exposed to DMF, with 13 000 workers potentially exposed for more than 20 h/week (NIOSH, 1983).

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[1] Data retrieval by J. Tickner from National Exposure Data Base, Health and Safety Executive (hse.gsi.gov.uk), 2000.

7. COMPARATIVE KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Available data indicate that DMF is readily absorbed following oral, dermal, and inhalation exposure in both humans and animals. The rate of dermal absorption was estimated to be 57 mg/cm^2 per 8 h in a rat tail model. DMF is metabolized primarily in the liver and is relatively rapidly excreted as metabolites in urine, primarily as *N*-(hydroxymethyl)-*N*-methylformamide (HMMF).

7.1 Experimental animals

The major metabolic pathway for DMF in mammalian species is oxidation by the cytochrome P-450-dependent mixed-function oxidase system to HMMF (Figure 1). This can generate NMF and formaldehyde (see review by Gescher, 1993). Further cytochrome P-450-mediated oxidation of NMF and/or HMMF results in the formation of *S*-(*N*-methylcarbamoyl)glutathione (SMG), the conjugate of the presumed reactive (toxic) intermediate, methyl isocyanate, excreted *in vivo* as *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC). Results of studies with liver microsomes from acetone-treated rats (Mráz et al., 1993; Chieli et al., 1995) and mice (Chieli et al., 1995) and with reconstituted enzyme systems indicate that cytochrome P-450 2E1 mediates the metabolism of DMF to HMMF and, subsequently, to the proposed reactive intermediate, methyl isocyanate.

The most informative of the toxicokinetic and metabolic studies relevant to consideration of inter-species and dose-related variations in toxicokinetics and metabolism include investigations following oral administration to rats and inhalation exposure of rats, mice, and monkeys.[2]

In female Sprague-Dawley rats administered a single oral dose of 100 mg ^{14}C-labelled DMF/kg body weight on day 12 or 18 of pregnancy, 60–70% of the radioactivity was excreted in urine and 3–4% in faeces at 48 h (Saillenfait et al., 1997). Approximately 4% of the dose was present in the liver at 0.5 h after dosing at both gestation times, with 8 and 13% in the gastrointestinal tract (stomach and intestine) and 0.7 and 0.8% in

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[2] In early studies, HMMF was not reported, since it degraded to NMF thermolytically in GLC conditions; hence, in early investigations, NMF = HMMF + NMF. HMMF is stable in aqueous solutions of neutral or mildly acidic pH but undergoes thermal decomposition to NMF during routine GC analysis. Therefore, it was first identified as NMF.
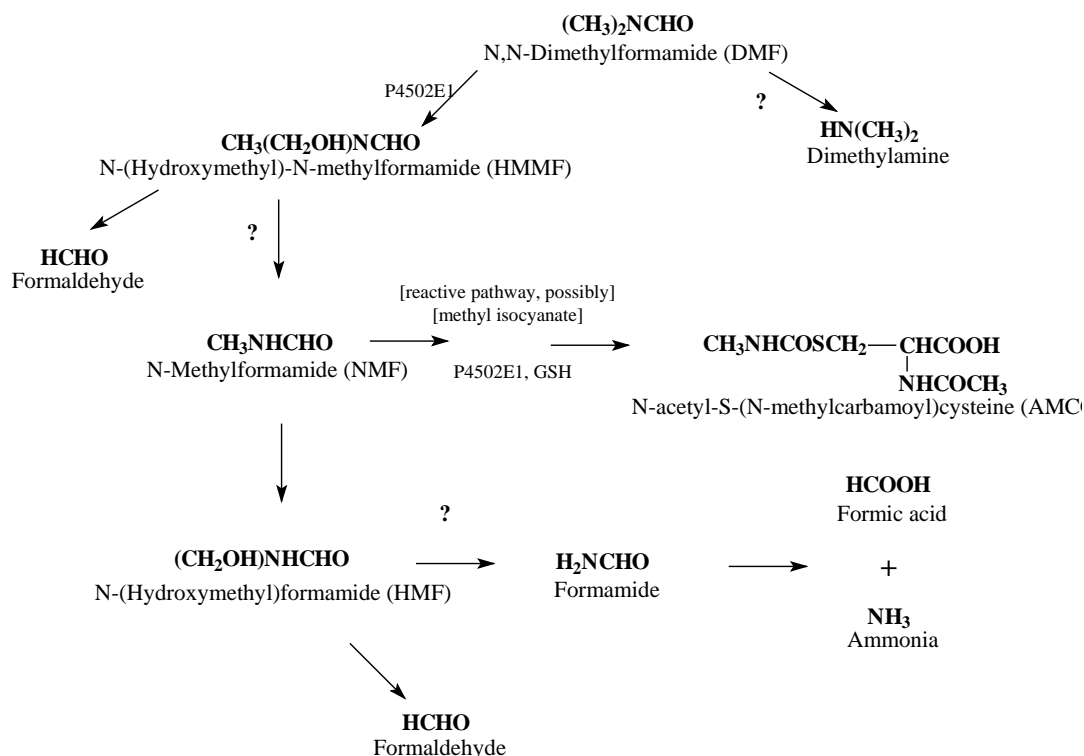
$(CH_3)_2NCHO$
N,N-Dimethylformamide (DMF)

P4502E1   ?

$CH_3(CH_2OH)NCHO$ $HN(CH_3)_2$
N-(Hydroxymethyl)-N-methylformamide (HMMF)   Dimethylamine

?

$HCHO$
Formaldehyde

[reactive pathway, possibly]
[methyl isocyanate]

CH_3NHCHO $CH_3NHCOSCH_2$—$CHCOOH$
N-Methylformamide (NMF)   P4502E1, GSH $NHCOCH_3$
N-acetyl-S-(N-methylcarbamoyl)cysteine (AMC

$HCOOH$
Formic acid

?

$(CH_2OH)NHCHO$ H_2NCHO +
N-(Hydroxymethyl)formamide (HMF)   Formamide
NH_3
Ammonia

$HCHO$
Formaldehyde

Fig. 1. Biotransformation of DMF (adapted from WHO, 1991; Gescher, 1993).

the kidneys, respectively. Plasma radioactivity was relatively constant from 0.5 to 4 h after dosing (approximately 0.4–0.5% of the dose) but declined rapidly thereafter. By 48 h, only the liver (0.5 and 0.6%) and intestine (0.2 and 0.3%) retained any significant activity. In animals exposed on day 12 of gestation, approximately 1.5% of the dose was present in the uterus, placenta, embryo, and amniotic fluid at between 0.5 and 4 h, which rapidly declined to less than 0.1% at 24 h. In rats exposed on day 18 of gestation, fetal tissues accounted for 6% of the administered dose. HPLC analysis performed at intervals from 1 to 24 h indicated that unchanged DMF and metabolites were readily transferred to the embryonic and fetal tissues, where levels were generally equal to those in maternal plasma. The parent compound accounted for most of the radioactivity until 4–8 h and then decreased.

Levels of parent compound and metabolites were determined in the plasma, amniotic fluid, placenta, and embryo in this investigation. Unchanged DMF initially accounted for the major proportion of radiolabelled carbon in the plasma or tissues, 61–77% for the first 4 h and 73–93% for the first 8 h after treatment on days 12 and 18, respectively. The decline in DMF levels corresponded with an increase in the levels of HMMF and NMF. HMMF accounted for 40–47% of ^{14}C at 8 h (day 12) and for 41–55% at 16 h (day 18). The equivalent figures for NMF were 9–13% and 16–18%, respectively. The amounts of AMCC and formamide in plasma or tissues were <4% of total radioactivity at all time points (Saillenfait et al., 1997). Other investigators have reported that DMF also crosses the placenta of pregnant rats after inhalation exposure (Sheveleva et al., 1977; Shumilina, 1991).

In another of the few recent investigations, levels of DMF, NMF, and HMMF were determined in the blood and urine of B6C3F1 mice and Crl:CD BR rats exposed to 10, 250, or 500 ppm (30, 750, or 1500 mg/m^3) for either single exposures of 1, 3, or 6 h or for 6 h/day, 5 days/week, for 2 weeks (Hundley et al., 1993a). The values for area under the plasma concentration curve (AUC) for DMF increased disproportionately in comparison with exposure, following single 6-h exposures to 250 and 500 ppm (750 and 1500 mg/m^3) (8- and 28-fold for rats and mice, respectively), while levels of NMF in the blood did not increase, which the authors considered to be indicative of saturation of metabolism of DMF. In contrast, multiple exposures increased the capacity of both rats and mice to metabolize DMF; repeated exposures to 500 ppm (1500 mg/m^3) resulted in a 3- and 18-fold reduction in AUC values for rats and mice, respectively. Peak plasma levels for NMF were elevated. HMMF represented over 90% of the total of DMF and determined metabolites.

In a similar investigation, DMF, NMF, and HMMF in blood and urine were determined in male and female cynomolgus monkeys exposed to 30, 100, or 500 ppm (90, 300, or 1500 mg/m^3) for 6 h/day, 5 days/week, for 13 weeks (Hundley et al., 1993b). The values for the AUC increased disproportionally between 100 and 500 ppm (300 and 1500 mg/m^3) (19- to 37-fold in males and 35- to 54-fold in females), data consistent with saturation of metabolism. However, there was no corresponding decrease in NMF levels; rather, they increased proportionally with increases in exposure concentrations. For each concentration, AUC values, peak plasma concentration, and plasma half-lives were consistent throughout the duration of exposure. HMMF was the main urinary metabolite (56–95%), regardless of exposure level or duration of exposure. DMF was not readily excreted in the urine, and NMF was more prevalent in plasma than in urine, suggesting that it was metabolized to compounds not determined in the study.

In comparative analyses of the two studies, the authors indicated that toxicokinetic differences may, in part, contribute to the observed species differences in toxicity. The AUC values and peak plasma levels for DMF for rats and mice following a single 500 ppm (1500 mg/m^3) exposure are substantially greater than the respective values in monkeys following a similar exposure. Whereas repeated exposures to 500 ppm (1500 mg/m^3) in rats and mice enhanced metabolism, as indicated by diminished AUC values for DMF and increased plasma concentrations of NMF, this effect was not clearly demonstrated in monkeys.

Results of the more recent study in rats were qualitatively similar to earlier investigations in which plasma DMF and "NMF" levels were determined in the plasma of rats exposed to DMF by inhalation for single 3- or 6-h exposures (Kimmerle & Eben, 1975a; Lundberg et al., 1983). Results of several of these earlier studies were also suggestive that at very high concentrations, DMF inhibits its own biotransformation. For example, 3 h following a single 4-h inhalation exposure of rats to 1690 or 6700 mg/m^3, levels of NMF in blood were lower in the higher exposure group (Lundberg et al., 1983). Similarly, Kimmerle & Eben (1975a) reported lower concentrations of NMF in the blood of rats exposed to 6015 mg/m^3 for 3 h than in rats exposed to 513 mg/m^3 for 6 h.

In a number of early studies, the effects of co-administration of ethanol on blood concentrations of DMF, NMF, ethanol, and acetaldehyde were investigated. Although there were variations in results depending on dose, time interval between administration of DMF and ethanol, and routes of exposure, there were increases in concentrations of DMF, NMF, ethanol, or acetaldehyde in blood upon co-exposure. These results may be attributable to inhibition by DMF of the activity of alcohol dehydrogenase observed both *in vitro* and *in vivo* (Eben & Kimmerle, 1976; Hanasono et al., 1977; Sharkawi, 1979) and of aldehyde dehydrogenase observed *in vivo* (Elovaara et al., 1983).

7.2   Humans

7.2.1   Studies in human volunteers

There were a number of early investigations in which the parent compound and some metabolites (not including that of the putatively toxic pathway) in blood and urine were determined in volunteers following short-term exposure to DMF (26 or 87 ppm [78 or 261 mg/m^3] for 4 h or 4 h/day for 5 days) (Kimmerle & Eben, 1975b). Results of these investigations indicated that DMF was rapidly excreted (the majority in 24 h), primarily as HMMF. Results of an additional early study in volunteers indicated that co-exposure to ethanol had a "slight influence" on the metabolism of DMF in volunteers receiving 19 g of ethanol 10 min prior to exposure to 82 ppm (246 mg/m^3) DMF for 2 h, based on lower concentrations of NMF in blood upon co-exposure. Contrary to the results in animals, there were no significant differences in the blood levels of ethanol and acetaldehyde upon co-exposure, which the authors attributed to the relatively low concentrations of DMF (Eben & Kimmerle, 1976).

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In a recent study in which the product of the putatively toxic pathway of metabolism (AMCC) was determined, 10 volunteers were exposed to 10, 30, or 60 mg DMF/m^3, for either single 8-h exposures or five daily exposures of 30 mg/m^3 (Mráz & Nohová, 1992a, 1992b). Urine was collected for 5 days and analysed for DMF, HMMF, HMF, and AMCC. In a separate protocol, three volunteers ingested 20 mg AMCC dissolved in water, and metabolites were determined for a period of 8 h after exposure. After single exposure to 30 mg/m^3, the proportions of metabolites eliminated in the urine were 0.3% parent compound, 22.3% HMMF, 13.2% HMF, and 13.4% AMCC. The half-times of excretion for these various metabolites were approximately 2, 4, 7, and 23 h, respectively. In contrast to this slow elimination after exposure to DMF, AMCC was rapidly eliminated after ingestion of AMCC, with a half-time of 1 h. These results were considered to be consistent with rate-limiting reversible protein binding of a reactive metabolic intermediate of DMF, possibly methyl isocyanate. Following repeated exposures, AMCC accumulated in urine. Although quantitative data were not presented, urinary elimination 16 h following the fifth exposure was approximately 14% HMMF, 32% HMF, and 54% AMCC.

7.2.2 Occupational environment

Exposure in the occupational environment may occur through both the dermal and inhalation routes. Lauwerys et al. (1980) reported that dermal absorption was more important than inhalation in the overall exposure, in the absence of personal protective devices.

There have been a number of reports of levels of DMF and metabolites in the blood and/or urine of workers. With the exception of more recent studies involving personal air sampling (Wrbitzky & Angerer, 1998),[1] few provide reliable quantitative data on relationship with exposure, though still not accounting for additional dermal exposure. Results of such studies have confirmed, however, the presence of AMCC (the product of the putatively toxic metabolic pathway) in the urine of workers.

Wrbitzky & Angerer (1998) noted a weak association between the concentration of DMF in workplace air and urinary concentration of NMF. Kawai et al. (1992) considered the relationship to be linear. In 116 workers exposed to TWA concentrations of 0.2, 0.4, 0.6, 3.9, or

9.1 ppm (0.6, 1.2, 1.8, 11.7, or 27.3 mg/m^3), the corresponding concentrations of NMF in urine were 0.7, 0.9, 2.6, 7.8, and 19.7 mg/litre.

Mráz et al. (1989) reported the detection of HMMF in urine samples from 12 DMF-exposed workers (extent of exposure not specified). Casal Lareo & Perbellini (1995) reported that AMCC accumulated throughout the work week in the urine of workers exposed to approximately 3–8 ppm (9–24 mg/m^3). Sakai et al. (1995) reported that levels of urinary AMCC remained constant over consecutive work days and increased after the end of exposure, with the peak concentration observed at 16–40 h after the end of exposure. Kafferlein[1] reported that urinary NMF concentrations were highest in post-shift samples, with a median half-time of 5.1 h. Concentrations of urinary AMCC reached a steady state 2 days after the beginning of exposure, with a half-time greater than 16 h.

7.2.3 Other relevant data

Angerer et al. (1998) reported that haemoglobin from individuals occupationally exposed to DMF contained *N*-carbamoylated valine residues derived from methyl isocyanate, the likely precursor of AMCC. The metabolism of DMF to HMMF by human liver microsomes *in vitro* has also been demonstrated. The addition of an antibody against rat liver cytochrome P-450 2E1 to the incubation mixture strongly inhibited DMF metabolism (Mráz et al., 1993).

7.3 Interspecies comparisons

In one of the few identified studies in which the product of the putatively toxic metabolic pathway (i.e., AMCC) was determined in animal species, Mráz et al. (1989) reported data on metabolites of DMF (DMF, HMMF, "HMF," AMCC) in 72-h urine samples following intraperitoneal administration of 0.1, 0.7, or 7 mmol/kg body weight to mice, rats, and hamsters. In addition, 10 healthy volunteers (5 males, 5 females) were exposed for 8 h to 20 ppm (60 mg/m^3). (The mean of the amount of DMF absorbed via the lung was reported to be half of the lowest dose administered in rodents.) Urine was collected and analysed for the same metabolites at 2- to 8-h intervals for 8 h for 4–5 days. The proportion of the total metabolites eliminated as AMCC was greatest in the rat (1.7–5.2%) and less in the hamster (1.5–1.9%) and mouse (1.1–1.6%). In rats exposed to the highest dose, excretion of DMF metabolites (including AMCC) was delayed. There was no clear dose-related variation in proportion of the metabolites determined excreted as AMCC in the animal species. In humans, a greater proportion of the absorbed dose (14.5%) following

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[1] Also written comments provided by H. Kafferlein, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander University Erlangen-Nuremberg, Germany, 2000.

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inhalation was present as AMCC in the urine. Absorption through the skin was not taken into account.

8. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

8.1 Single exposure

Following oral, dermal, inhalation, or parenteral administration, the acute toxicity of DMF in a number of species is low. Lethal doses are generally in the g/kg body weight range for oral, dermal, and parenteral routes and in the g/m^3 range for inhalation exposure. Clinical signs following acute exposure include general depression, anaesthesia, loss of appetite, loss of body weight, tremors, laboured breathing, convulsions, haemorrhage at nose and mouth, liver injury, and coma preceding death. Where protocols included histopathological examination, damage was observed primarily in the liver (WHO, 1991). In the rat, oral LD_{50}s range from 3000 to 7170 mg/kg body weight, dermal LD_{50}s range from 5000 to >11 520 mg/kg body weight, and inhalation LC_{50}s range from 9432 to 15 000 mg/m^3 (WHO, 1991).

8.2 Irritation and sensitization

Standard tests for dermal irritation by DMF have not been identified, and data on its sensitization potential are conflicting. Hence, only limited conclusions can be drawn concerning the potential of DMF to induce these effects.

IARC (1999), WHO (1991), and Kennedy (1986) reviewed the effects of DMF on the skin and eyes and reported only mild to moderate effects. A single application of neat DMF to the shaved skin of mice at 1–5 g/kg body weight (precise exposure conditions not specified) produced slight transient skin irritation at 2.5–5 g/kg body weight, while similar treatment of rabbits at up to 0.5 g/kg body weight was without effect (Kennedy, 1986; WHO, 1991). Repeated (15- or 28-day) applications of 1–2 g/kg body weight did not induce marked local effects on the skin of rats or rabbits. The instillation of neat or 50% aqueous DMF into the rabbit eye produced moderate corneal injury and moderate to severe conjunctivitis, with some damage still evident 14 days later (Kennedy, 1986; WHO, 1991; IARC, 1999).

In a murine local lymph node assay predictive for identification of contact allergens, cell proliferation (based on [3H]thymidine incorporation in lymph nodes) was significantly increased (324 vs. 193 decompositions per minute per lymph node in exposed and control groups, respectively) in mice (strain not specified) receiving a daily topical application of 25 µl on the dorsum of both ears for 3 consecutive days (Montelius et al., 1996). In subsequent assays, thymidine incorporation in DMF-exposed mice was up to 3-fold higher than in naive mice. However, statistical analyses were not presented, and the increase was not considered to be significant (Montelius et al., 1998). The naive (non-treated) mice were included in the protocol to measure the magnitude of vehicle (DMF)-induced proliferation. In contrast, Kimber & Weisenberger (1989) detected no difference in proliferation in a lymph node assay in which lymph node cells from DMF (the solvent)-exposed mice were compared with those from naive mice.

8.3 Short-term exposure

While there have been a number of primarily early short-term studies, these have generally been restricted to examination of specific effects following exposure to single dose levels. They are not additionally informative concerning the toxicity of DMF but confirm a range of effects in the liver, which, when considered collectively across studies, are consistent with a profile in rats of alterations in hepatic enzymes and increases in liver weight at lowest concentrations and degenerative histopathological changes, cell death, and increases in serum hepatic enzymes at higher concentrations. Although results of a short-term study in monkeys also indicate that this species is less sensitive to the effects of DMF than rats, the protocol had only one exposure concentration, and there were only two monkeys in the experiment (Hurtt et al., 1991).

In the only short-term investigation in which a dose–response relationship for hepatic effects was characterized, there was a dose-related increase in liver to body weight ratio, significant at all levels of exposure, and in activity of uridine disphosphate glucuronosyl-transferase in male Wistar rats exposed for 2 weeks via drinking-water to approximately 0, 14, 70, or 140 mg/kg body weight per day (Elovaara et al., 1983). Such changes have not been observed at such low doses in more recent, longer-term studies.

Available data from acute and short-term studies also indicate that there are effects on metabolizing enzymes at very high doses (i.e., 475 mg/kg body weight per day and above administered subcutaneously to rats). These include glutathione metabolism (although reported changes at two different doses were not

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consistent) and decreases in hepatic microsomal P-450 content (Imazu et al., 1992, 1994; Fujishiro et al., 1996).

8.4   Medium-term exposure

Information on the incidences of lesions in the critical medium-term exposure studies is presented in Tables 2 and 3.

8.4.1   Inhalation

The NTP (1992a) carried out a subchronic bioassay in F344 rats, exposing males and females to 0, 50, 100, 200, 400, or 800 ppm (0, 150, 300, 600, 1200, or 2400 mg/m^3) for 6 h/day, 5 days/week, for 13 weeks. The authors designated 200 ppm (600 mg/m^3) as a no-observed-adverse-effect level (NOAEL) for both sexes, based upon the absence of histopathological lesions in liver. Minimal to moderate hepatocellular necrosis in both sexes was observed at 400 and 800 ppm (1200 and 2400 mg/m^3), with the lesion more severe in females. However, in males, both the absolute and relative weights of liver were significantly increased at 100 ppm (300 mg/m^3) and greater, although there was no clear dose–response, as weights declined at the highest dose. Serum cholesterol was increased at all levels of exposure; again, there was no clear dose–response. In males at day 24, there was a dose-related increase in serum alanine aminotransferase (ALT) (significant at all levels of exposure); however, at day 91, the increase was significant only at 400 ppm (1200 mg/m^3). At day 91, there was also a dose-related increase in serum sorbitol dehydrogenase in males (significant at 200 ppm [600 mg/m^3]). In females, relative liver weight was significantly increased at all levels of exposure, with the weight declining at the highest dose. Serum cholesterol was significantly increased at all levels of exposure in females, with no clear dose–response. At day 91 in females, serum sorbitol dehydrogenase and isocitrate dehydrogenase were significantly increased at 200 ppm (600 mg/m^3) and greater.

Craig et al. (1984) exposed male and female F344 rats to 0, 150, 300, 600, or 1200 ppm (0, 450, 900, 1800, or 3600 mg/m^3) for 6 h/day, 5 days/week, for 12 weeks. There were few overt signs of toxicity. Body weight was significantly decreased in both sexes at the highest dose. There were some changes in clinical chemistry and haematological parameters at the highest doses. In males, serum cholesterol was significantly increased at the highest concentration only. Serum alkaline phosphatase (AP) was reduced in a dose-related manner, beginning at 300 ppm (900 mg/m^3). In females, cholesterol was significantly increased at 600 and 1200 ppm (1800 and 3600 mg/m^3). In contrast to males, serum AP

was increased in a dose-related manner (significant at the two highest concentrations). Data on organ weights were not presented. Histopathological changes were observed in the liver at the highest doses, were "barely discernible" at 300 ppm (900 mg/m^3), and were not observed at 150 ppm (450 mg/m^3). The lowest-observed-adverse-effect concentration (LOAEC) for both sexes is 300 ppm (900 mg/m^3), based upon slight histopathological changes in the liver (no-observed-effect concentration [NOEC] = 150 ppm [450 mg/m^3]).

B6C3F1 mice were exposed to 0, 50, 100, 200, 400, or 800 ppm (0, 150, 300, 600, 1200, or 2400 mg/m^3) for 6 h/day, 5 days/week, for 13 weeks (NTP, 1992a). Relative liver weight was significantly increased in both sexes at all levels of exposure, although the dose–response was not clear. Absolute liver weight was significantly increased in females at all dose levels, although the dose–response was not clear. Centrilobular hepatocellular hypertrophy (minimal to mild) was observed in all exposed males and in females at 100 ppm (300 mg/m^3) and higher (lowest-observed-effect concentration [LOEC] = 50 ppm [150 mg/m^3]).

Craig et al. (1984) exposed B6C3F1 mice to 0, 150, 300, 600, or 1200 ppm (0, 450, 900, 1800, or 300 mg/m^3) for 6 h/day, 5 days/week, for 12 weeks. Mortality was 10% at 600 ppm (1800 mg/m^3) and 40% at 1200 ppm (3600 mg/m^3). No adverse effects on haematology or clinical chemistry were observed. Hepatic cytomegaly was observed in all exposed mice; the incidence and severity were related to dose (LOEC = 150 ppm [450 mg/m^3]).

Hurtt et al. (1992) exposed three male and three female cynomolgus monkeys to 0, 30, 100, or 500 ppm (0, 90, 300, or 1500 mg/m^3) for 6 h/day, 5 days/week, for 13 weeks. Two males were maintained for a further 13-week observation period after exposure had ceased. The protocol included microscopic examination of a comprehensive range of organ tissues in all animals. Sperm morphology and vaginal cytology were also evaluated in all animals. There were no overt signs of toxicity and no effects on body weight gain, haematology, clinical chemistry, urinalysis, organ weights, or histopathological effects attributable to DMF in cynomolgus monkeys exposed to up to 500 ppm (1500 mg/m^3), leading the authors to conclude that the monkey is much less sensitive than the rat or mouse (Hurtt et al., 1992).

The other inhalation studies are either poorly reported or limited in their scope (Massmann, 1956; Clayton et al., 1963; Cai & Huang, 1979; Arena et al., 1982). One group of investigators reported effects on the

Table 2: Effect levels and benchmark concentrations for DMF, inhalation exposure.

Study (reference)	Effect level	Data for calculating benchmark concentration		Benchmark concentration		Goodness of fit
		Concentration	Response	Parameter estimates[a,b]		
Medium-term exposure						
B6C3F1 mice 10 males and 10 females per group 0, 50, 100, 200, 400, 800 ppm, 6 h/day, 5 days/week, for 13 weeks (NTP, 1992a)	LOEC = 50 ppm, based upon increased relative liver weight in both sexes and hepatocellular hypertrophy in males	Male, incidence (severity) of centrilobular hepatocellular hypertrophy: control / 50 ppm / 100 ppm / 200 ppm / 400 ppm / 800 ppm	0/10 / 4/10 (1.8) / 9/10 (1.3) / 10/10 (2.0) / 10/10 (2.0) / 10/10 (2.0)	BMC_{05} = 8.5 ppm excluding 400 and 800 ppm groups Adjusted BMC_{05} = 1.51 ppm	95% LCL_{05} = 2.5 ppm excluding 400 and 800 ppm groups Adjusted 95% LCL_{05} = 0.44 ppm	Chi-square (1) = 0.004 P-value = 0.99
		Female, incidence (severity) of centrilobular hepatocellular hypertrophy: control / 50 ppm / 100 ppm / 200 ppm / 400 ppm / 800 ppm	0/10 / 0/10 / 10/10 (1.3) / 10/10 (1.9) / 10/10 (2.0) / 10/10 (2.0)	BMC_{05} = 17.9 ppm excluding 200, 400, and 800 ppm groups Adjusted BMC_{05} = 3.19 ppm excluding 200, 400, and 800 ppm groups	95% LCL_{05} = 8.1 ppm excluding 200, 400, and 800 ppm groups Adjusted 95% LCL_{05} = 1.45 ppm excluding 200, 400, and 800 ppm groups	Chi-square (1) = 7.5 P-value = 0.01
Long-term exposure/carcinogenicity assays						
Rat, Crl:CD BR 87 males and 87 females per group 0, 25, 100, 400 ppm, 6 h/day, 5 days/week, for 2 years (Malley et al., 1994)	LOEC = 100 ppm, based upon a signif- icant increase in centrilobular hepatocellular hypertrophy (both sexes), hepatic accumulation of lipo- fuscin/haemosiderin (both sexes), and hepatic single-cell necrosis (females only) NOEC = 25 ppm	females, hepatic accumulation of lipofuscin/haemosiderin: control (n = 60) / 25 ppm (n = 59) / 100 ppm (n = 59) / 400 ppm (n = 62)	8% / 7% / 22% ($P < 0.05$) / 61% ($P < 0.05$)	BMC_{05} = 37.0 ppm Adjusted BMC_{05} = 6.61 ppm	95% LCL_{05} = 19.8 ppm Adjusted 95% LCL_{05} = 3.54 ppm	Chi-square (1) = 1.01 P-value = 0.31
		males, hepatic accumulation of lipofuscin/haemosiderin: control (n = 57) / 25 ppm (n = 59) / 100 ppm (n = 58) / 400 ppm (n = 60)	4% / 4% / 17% ($P < 0.05$) / 58% ($P < 0.05$)	BMC_{05} = 41.4 ppm Adjusted BMC_{05} = 7.39 ppm	95% LCL_{05} = 21.9 ppm Adjusted 95% LCL_{05} = 3.91 ppm	Chi-square (1) = 0.84 P-value = 0.36
		males, relative liver weight: control (n = 17) / 25 ppm (n = 19) / 100 ppm (n = 21) / 400 ppm (n = 26)	2.87 / 2.81 / 3.28 / 3.58 ($P < 0.05$)	BMC_{05} = 44.5 ppm Adjusted BMC_{05} = 7.95 ppm	95% LCL_{05} = 23.7 ppm Adjusted 95% LCL_{05} = 4.23 ppm	$F(1,79)$ = 2.09 P-value = 0.15
		males, hepatic foci of alterations (clear cell): control (n = 57) / 25 ppm (n = 59) / 100 ppm (n = 58) / 400 ppm (n = 60)	11% / 8% / 22% ($P < 0.05$) / 35% ($P < 0.05$)	BMC_{05} = 57.7 ppm Adjusted BMC_{05} = 10.3 ppm	95% LCL_{05} = 37.8 ppm Adjusted 95% LCL_{05} = 6.75 ppm	Chi-square (2) = 1.71 P-value = 0.42

Table 2 (contd).

Study (reference)	Effect level	Data for calculating benchmark concentration		Benchmark concentration		
		Concentration	Response	Parameter estimates[a,b]		Goodness of fit
		females, hepatic foci of alterations (clear cell): control ($n = 60$) 5% 25 ppm ($n = 59$) 5% 100 ppm ($n = 59$) 14% 400 ppm ($n = 62$) 24% ($P < 0.05$)		$BMC_{05} = 84.3$ ppm Adjusted $BMC_{05} = 15.1$ ppm	95% $LCL_{05} = 53.4$ ppm Adjusted 95% $LCL_{05} = 9.54$ ppm	Chi-square (2) = 0.77 P-value = 0.68
Rat, Crl:CD BR 87 males and 87 females per group 0, 25, 100, 400 ppm, 6 h/day, 5 days/week, for 2 years (Malley et al., 1994)	LOEC = 100 ppm, based upon a signifi-cant increase in centri-lobular hepatocellular hypertrophy (both sexes), hepatic accumulation of lipofuscin/haemosiderin (both sexes), and hepatic single-cell necrosis (females only) NOEC = 25 ppm	females, relative liver weight: control ($n = 22$) 3.12 25 ppm ($n = 14$) 3.43 100 ppm ($n = 12$) 3.33 400 ppm ($n = 23$) 3.86 ($P < 0.05$)		$BMC_{05} = 101.6$ ppm Adjusted $BMC_{05} = 18.1$ ppm	95% $LCL_{05} = 46.2$ ppm Adjusted 95% $LCL_{05} = 8.25$ ppm	$F(1,67) = 1.12$ P-value = 0.29
		males, centrilobular hepatocellular hypertrophy: control ($n = 57$) 0 25 ppm ($n = 59$) 0 100 ppm ($n = 58$) 5% ($P < 0.05$) 400 ppm ($n = 60$) 30% ($P < 0.05$)		$BMC_{05} = 118.7$ ppm Adjusted $BMC_{05} = 21.2$ ppm	95% $LCL_{05} = 56.4$ ppm Adjusted 95% $LCL_{05} = 10.1$ ppm	Chi-square (1) = 0.65 P-value = 0.42
		females, centrilobular hepatocellular hypertrophy: control ($n = 60$) 0 25 ppm ($n = 59$) 0 100 ppm ($n = 59$) 3% ($P < 0.05$) 400 ppm ($n = 62$) 40% ($P < 0.05$)		$BMC_{05} = 126.7$ ppm Adjusted $BMC_{05} = 22.6$ ppm	95% $LCL_{05} = 77.7$ ppm Adjusted 95% $LCL_{05} = 13.9$ ppm	Chi-square (1) = 0.13 P-value = 0.72
		females, hepatic single-cell necrosis: control ($n = 60$) 0 25 ppm ($n = 59$) 0 100 ppm ($n = 59$) 5% ($P < 0.05$) 400 ppm ($n = 62$) 18% ($P < 0.05$)		$BMC_{05} = 126.9$ ppm Adjusted $BMC_{05} = 22.7$ ppm	95% $LCL_{05} = 72.9$ ppm Adjusted 95% $LCL_{05} = 13.0$ ppm	Chi-square (1) = 0.78 P-value = 0.38
Mice, Crl:CD 1 (ICR)BR 78 males and 78 females per group 0, 25, 100, 400 ppm, 6 h/day, 5 days/week, for 18 months (Malley et al., 1994)	LOEC = 25 ppm, based upon centrilobular hepatocellular hyper-trophy (males), hepatic single-cell necrosis (males and females), and hepatic Kupffer cell hyperplasia/pigment accumulation (males)	females, hepatic single-cell necrosis: control ($n = 61$) 29% 25 ppm ($n = 63$) 44% ($P < 0.05$) 100 ppm ($n = 61$) 70% ($P < 0.05$) 400 ppm ($n = 63$) 76% ($P < 0.05$)		$BMC_{05} = 16.8$ ppm $BMC_{05} = 5.9$ ppm excluding 400 ppm group Adjusted $BMC_{05} = 3.00$ ppm $BMC_{05} = 1.05$ ppm excluding 400 ppm group	95% $LCL_{05} = 11.9$ ppm 95% $LCL_{05} = 4.1$ ppm excluding 400 ppm group Adjusted 95% $LCL_{05} = 2.13$ ppm 95% $LCL_{05} = 0.73$ ppm excluding 400 ppm group	Chi-square (2) = 9.7 P-value = 0.00 (Chi-square (1) = 0.02 P-value = 0.88)
		males, hepatic single-cell necrosis: control ($n = 60$) 24% 25 ppm ($n = 62$) 59% ($P < 0.05$) 100 ppm ($n = 60$) 68% ($P < 0.05$) 400 ppm ($n = 59$) 87% ($P < 0.05$)		$BMC_{05} = 10.8$ ppm Adjusted $BMC_{05} = 1.93$ ppm	95% $LCL_{05} = 7.8$ ppm Adjusted 95% $LCL_{05} = 1.39$ ppm	Chi-square (2) = 13.4 P-value = 0.00
		males, hepatic Kupffer cell hyperplasia/pigment accumulation: control ($n = 60$) 22% 25 ppm ($n = 62$) 52% ($P < 0.05$) 100 ppm ($n = 60$) 60% ($P < 0.05$) 400 ppm ($n = 59$) 86% ($P < 0.05$)		$BMC_{05} = 11.1$ ppm Adjusted $BMC_{05} = 1.98$ ppm	95% $LCL_{05} = 8.2$ ppm Adjusted 95% $LCL_{05} = 1.46$ ppm	Chi-square (2) = 7.5 P-value = 0.02

Table 2 (contd).

| Study (reference) | Effect level | Data for calculating benchmark concentration | | Benchmark concentration | | |
		Concentration	Response	Parameter estimates[a,b]		Goodness of fit
		females, hepatic Kupffer cell hyperplasia/pigment accumulation: control (n = 61) 51% 25 ppm (n = 63) 57% 100 ppm (n = 61) 71% (P < 0.05) 400 ppm (n = 63) 89% (P < 0.05)		BMC_{05} = 13.4 ppm Adjusted BMC_{05} = 2.39 ppm	95% LCL_{05} = 9.3 ppm Adjusted 95% LCL_{05} = 1.66 ppm	Chi-square (2) = 0.35 P-value = 0.84
Mice, Crl:CD 1 (ICR)BR 78 males and 78 females per group 0, 25, 100, 400 ppm, 6 h/day, 5 days/week, for 18 months (Malley et al., 1994)	LOEC = 25 ppm, based upon centrilobular hepatocellular hypertrophy (males), hepatic single-cell necrosis (males and females), and hepatic Kupffer cell hyperplasia/pigment accumulation (males)	males, centrilobular hepatocellular hypertrophy: control (n = 60) 0 25 ppm (n = 62) 8% (P < 0.05) 100 ppm (n = 60) 41% (P < 0.05) 400 ppm (n = 59) 52% (P < 0.05)		BMC_{05} = 18.9 ppm Adjusted BMC_{05} = 3.38 ppm BMC_{05} = 2.93 ppm excluding 400 ppm group	95% LCL_{05} = 15.3 ppm Adjusted 95% LCL_{05} = 0.95 ppm 95% LCL_{05} = 1.48 ppm excluding 400 ppm group	Chi-square (2) = 0.77 P-value = 0.00 (Chi-square (0) = 0.00 P-value = 1.00)
		females, centrilobular hepatocellular hypertrophy: control (n = 61) 0 25 ppm (n = 63) 6% 100 ppm (n = 61) 19% (P < 0.05) 400 ppm (n = 63) 54% (P < 0.05)		BMC_{05} = 25.1 ppm Adjusted BMC_{05} = 4.48 ppm	95% LCL_{05} = 19.9 ppm Adjusted 95% LCL_{05} = 3.55 ppm	Chi-square (2) = 0.39 P-value = 0.82
		males, relative liver weight: control (n = 31) 5.85 25 ppm (n = 42) 5.94 100 ppm (n = 38) 7.06 (P < 0.05) 400 ppm (n = 36) 7.80 (P < 0.05)		BMC_{05} = 65.6 ppm Adjusted BMC_{05} = 11.7 ppm	95% LCL_{05} = 37.5 ppm Adjusted 95% LCL_{05} = 6.69 ppm	$F(1,143)$ = 1.94 P-value = 0.17
		females, relative liver weight: control (n = 42) 5.59 25 ppm (n = 35) 5.71 100 ppm (n = 36) 5.99 400 ppm (n = 47) 6.35 (P < 0.05)		BMC_{05} = 144.7 ppm Adjusted BMC_{05} = 25.8 ppm	95% LCL_{05} = 76.3 ppm Adjusted 95% LCL_{05} = 13.6 ppm	$F(1,156)$ = 0.34 P-value = 0.56

[a] Adjusted from intermittent exposure (h/day, days/week) to continuous exposure.
[b] LCL = Lower confidence limit.

Table 3: Effect levels and benchmark doses for DMF, oral exposure.

Study (reference)	Effect level	Data for calculating benchmark dose		Benchmark dose		Goodness of fit
		Dose (mg/kg body weight per day)	Response	Parameter estimates		
Medium-term exposure						
Rat, Wistar 25 males and 25 females per group Dietary administration for 15 weeks (Becci et al., 1983)	LOEL = 69 mg/kg body weight per day, based upon a significant increase in relative liver weight in females at the two highest doses (NOEL = 20 mg/kg body weight per day)	males, relative liver weight: control (n = 25) 18 (n = 23) 61 (n = 25) 210 (n = 23)	4.30 ± 0.09 4.51 ± 0.11 4.59 ± 0.08 4.99 ± 0.10 ($P < 0.05$)	BMD_{05} = 23.1 mg/kg body weight per day	95% LCL_{05} = 12.7 mg/kg body weight per day	$F_{(1,92)}$ = 0.73 P-value = 0.39
		females, relative liver weight: control (n = 25) 20 (n = 25) 69 (n = 24) 235 (n = 24)	86 ± 0.06 89 ± 0.08 24 ± 0.12 ($P < 0.05$) 00 ± 0.12 ($P < 0.05$)	BMD_{05} = 35.9 mg/kg body weight per day	95% LCL_{05} = 15.7 mg/kg body weight per day	$F_{(1,94)}$ = 0.13 P-value = 0.72
Mouse, CD-1 30 males and 30 females per group dietary administration for 17 weeks (Becci et al., 1983)	LOEL = 96 mg/kg body weight per day, based upon statistically significant increase in relative liver weight in females (NOEL = 28 mg/kg body weight per day)	males, relative liver weight: control (n = 30) 22 (n = 28) 70 (n = 29) 246 (n = 29)	5.3 ± 0.1 5.6 ± 0.1 5.8 ± 0.1 6.6 ± 0.1 ($P < 0.01$)	BMD_{05} = 21.3 mg/kg body weight per day	95% LCL_{05} = 7.6 mg/kg body weight per day	$F_{(1,112)}$ = 1.17 P-value = 0.28
		females, relative liver weight: control (n = 30) 28 (n = 29) 96 (n = 29) 326 (n = 30)	5.1 ± 0.2 5.5 ± 0.1 5.9 ± 0.1 ($P < 0.01$) 6.6 ± 0.3 ($P < 0.01$)	BMD_{05} = 36.8 mg/kg body weight per day	95% LCL_{05} = 21.3 mg/kg body weight per day	$F_{(1,114)}$ = 0.14 P-value = 0.71

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liver of rats exposed to DMF vapour for 18 weeks at a concentration of just 7.3 ppm (21.9 mg/m^3) (no further details provided in the citation) (Cai & Huang, 1979). Myocardial changes occurred in rabbits exposed to 40 ppm (120 mg/m^3) for 50 days (Arena et al., 1982).

8.4.2   Oral

In a 90-day dietary study, Crl:CD rats were exposed to 0, 10, 50, or 250 mg/kg body weight per day (Haskell Laboratory, 1960; Kennedy & Sherman, 1986). Mild effects on the liver (enlargement of hepatic cells) and haematological effects (anaemia, leukocytosis) were observed at 50 mg/kg body weight per day; at the top dose of 250 mg/kg body weight per day, weight gain was reduced, and the animals had slight anaemia, leukocytosis, and liver cell enlargement. Although there was an apparent increase in serum cholesterol in both sexes at the highest dose, statistical analyses were not presented. The no-observed-effect level (NOEL) was 10 mg/kg body weight per day. The lowest-observed-effect level (LOEL) is 50 mg/kg body weight per day, based upon a significant increase in relative liver weight in males.

In a second study involving larger group sizes, a different strain (Wistar), and more comprehensive tissue examination, growth was inhibited but no tissue lesions were observed in rats administered DMF in the diet for 15 weeks (Becci et al., 1983). Males received 0, 18, 61, or 210 mg/kg body weight per day, and females received 0, 20, 69, or 235 mg/kg body weight per day. The LOEL is 69 mg/kg body weight per day, based upon a significant increase in relative liver weight in females at the two highest doses (NOEL = 20 mg/kg body weight per day).

In the corresponding study in CD-1 mice involving dietary administration (males: 0, 22, 70, or 246 mg/kg body weight per day; females: 0, 28, 96, or 326 mg/kg body weight per day) for 17 weeks, there were no overt signs of toxicity and no notable effects on blood morphology, blood biochemistry, or urinary parameters (Becci et al., 1983). Microscopic examination of an extensive range of organ tissues revealed only mild effects on the liver in the majority of high-dose males and females. There was a dose-related increase in relative liver weight at all dose levels, although this was statistically significant only in the mid- and high-dose females and in the high-dose males. On this basis, the LOEL is 96 mg/kg body weight per day, based upon a significant increase in relative liver weight in females (NOEL = 28 mg/kg body weight per day).

In a submission to the US EPA Office of Toxic Substances, BASF (1984) reported that there were no adverse effects observed in beagle dogs (four males and four females per group) administered 0, 1.4, 7.0, or 34.8 mg/kg body weight per day (NOEL) in the diet for 13 weeks. The protocol included measurement of food consumption, measurement of body weight gain, hearing tests, ophthalmoscopic examination, clinical laboratory investigations, measurement of organ weights, and histopathological observations.

8.5   Long-term exposure and carcinogenicity

Information on the incidences of lesions in critical long-term studies is presented in Tables 2 and 3.

8.5.1   Inhalation

Malley et al. (1994) exposed Crl:CD BR rats for 6 h/day, 5 days/week, to 0, 25, 100, or 400 ppm (0, 75, 300, or 1200 mg/m^3) DMF vapour for 24 months. There were no overt signs of toxicity other than a reduction in weight gain in the rats exposed at 400 ppm (1200 mg/m^3) and, to a lesser extent and towards the end of the study, in males exposed at 100 ppm (300 mg/m^3). Haematological findings were normal, as were urinary analyses. There was a concentration-related increase in serum sorbitol dehydrogenase activity (indicative of hepatic effects) in the male and female rats at 100 and 400 ppm (300 and 1200 mg/m^3). Relative liver weights were increased in both sexes at 400 ppm (1200 mg/m^3), and microscopic examination revealed hepatic lesions (centrilobular hepatocellular hypertrophy, lipofuscin/haemosiderin accumulation, clear cell foci, and single-cell necrosis in males and high-dose females and focal cystic degeneration in males) at 100 and 400 ppm (300 and 1200 mg/m^3). Microscopic examination of an extensive range of tissues from the high-dose animals (and of selected tissues from the lower dose groups) revealed no other treatment-related lesions except in females, in which there was an increased incidence of uterine endometrial stromal polyps (1.7%, 5.1%, 3.4%, and 14.8% for control, low-, mid-, and high-dose females, respectively). Historical control data from the same laboratory indicated a highly variable incidence of endometrial stromal polyps (2–15% for 14 control groups, average 6.6%). The investigators concluded that DMF was not carcinogenic to rats under the conditions of exposure. The LOEC was 100 ppm (300 mg/m^3) (NOEC = 25 ppm [75 mg/m^3]), based upon a significant increase in centrilobular hepatocellular hypertrophy (both sexes), significant increase in hepatic accumulation of lipofuscin/haemosiderin (both sexes), and hepatic single-cell necrosis (females only).

Mice [Crl:CD 1 (ICR)BR] were exposed to 0, 25, 100, or 400 ppm (0, 75, 300, or 1200 mg/m^3) DMF for 6 h/day, 5 days/week, for 18 months (Malley et al., 1994). Haematological observations were normal. Relative liver weight was significantly increased at the two highest concentrations in males. Microscopic alterations in liver were observed at all levels of exposure. The authors concluded that DMF was not carcinogenic to mice under the conditions of the bioassay. The LOEC is 25 ppm (75 mg/m^3), based upon centrilobular hepatocellular hypertrophy (males), hepatic single-cell necrosis (males and females), and hepatic Kupffer cell hyperplasia/pigment accumulation (males).

8.5.2   Oral

An inadequate carcinogenicity study involving the administration of DMF in the drinking-water of BD rats at approximately 10 or 20 mg/kg body weight per day for 500 or 250 days, respectively, provided no evidence of tumour formation, although the extent of tissue examination was not specified (Druckrey et al., 1967). In female Mongolian gerbils administered DMF in the drinking-water at concentrations of 1.0–6.6% (around 5–40 mg/kg body weight per day) for up to 200 days, there were many early deaths at concentrations of 1.7% (around 7–11 mg/kg body weight per day) and above, and all DMF-exposed groups had liver degeneration and kidney congestion (Llewellyn et al., 1974).

8.5.3   Injection

In a study in hamsters investigating the carcino-genic activity of aflatoxins, there was no mention of any tumours in the DMF-treated controls. These animals (five males and five females) received weekly intra-peritoneal injections of 0.1 ml of a 50% DMF solution (equivalent to approximately 47 mg DMF/kg body weight per injection) for 6–8.5 months and were then maintained untreated until they died (average life span 19 months) (Herrold, 1969). Although there were no increases in tumours following repeated intraperitoneal injections of DMF to rats for 10 weeks in a study reported in a secondary source, available information was inadequate to permit critical review (Kommineni, 1973).

8.6   Genotoxicity and related end-points

The following discussion is limited to results of assays for gene mutation and cytogenesis, i.e., those assays in which the end-points are most relevant to the assessment of DMF with respect to human health.

The results of assays for gene mutation *in vitro* were almost entirely negative. Of 20 identified assays in

Salmonella, results were negative in 18 (Green & Savage, 1978; Purchase et al., 1978; Baker & Bonin, 1981; Brooks & Dean, 1981; Garner et al., 1981; Gatehouse, 1981; Ichinotsubo et al., 1981; MacDonald, 1981; Martire et al., 1981; Nagao & Takahashi, 1981; Richold & Jones, 1981; Rowland & Severn, 1981; Simmon & Shepherd, 1981; Skopek et al., 1981; Venitt & Crofton-Sleigh, 1981; Antoine et al., 1983; Falck et al., 1985; Mortelmans et al., 1986), and two had equivocal results (Hubbard et al., 1981; Trueman, 1981). Results in six assays in *Escherichia coli* were all negative (Gatehouse, 1981; Matsushima et al., 1981; Mohn et al., 1981; Thomson, 1981; Venitt & Crofton-Sleigh, 1981; Falck et al., 1985).

Although fewer assays for cytogenetic effects and genotoxicity *in vitro* were identified than for gene mutation, results were also predominantly negative. In assays for chromosomal aberrations (CAs), results were negative for human lymphocytes (Antoine et al., 1983) and Chinese hamster ovary (CHO) (Natarajan & van Kesteren-van Leeuwen, 1981) and weakly positive in human peripheral lymphocytes (Koudela & Spazier, 1979). In three mouse lymphoma assays, results were negative (Jotz & Mitchell, 1981; Mitchell et al., 1988; Myhr & Caspary, 1988) and one was weakly positive (McGregor et al., 1988). Results of *in vitro* tests for sister chromatid exchange (SCE) were negative in three assays in CHO (Evans & Mitchell, 1981; Natarajan & van Kesteren-van Leeuwen, 1981; Perry & Thomson, 1981) and one in human lymphocytes (Antoine et al., 1983). Assays for unscheduled DNA synthesis (UDS) were negative in human fibroblasts (Agrelo & Amos, 1981; Robinson & Mitchell, 1981), mouse hepatocytes (Klaunig et al., 1984), and HeLa cells (Martin & McDermid, 1981), while in assays in rat hepatocytes, results were both negative (Ito, 1982) and positive (Williams, 1977). Results of assays for DNA repair in mouse (McQueen et al., 1983) and hamster (McQueen et al., 1983) hepatocytes were also negative. An assay for DNA repair in human hepatocytes had negative results (McQueen et al., 1988).

The database for genotoxicity studies *in vivo* is more limited than that for *in vitro* studies.

In two adequate assays for micronucleus induc-tion, results were negative (Kirkhart, 1981; Antoine et al., 1983). In the latter study, dose levels were too widely spaced, and the top dose was 2000 mg/kg body weight. Results were also negative in two assays in which there were no positive controls (Salamone et al., 1981; Tsuchimoto & Matter, 1981). It should be noted that Salamone et al. (1981) observed no effect at doses up to 80% of the LD$_{50}$. An assay in which an increase in

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micronuclei was observed in bone marrow of mice was reported only as an abstract (Ye, 1987), although a dose–response was not clear. Although six dose levels were included in the protocol, the highest dose was only 20 mg/kg body weight (oral LD_{50} values in laboratory animals range from 2000 to 7000 mg/kg body weight).

Negative results were reported in assays for chromosomal damage in bone marrow of rats (Sheveleva et al., 1979; McGregor, 1981) and dominant lethal assays in rats (Lewis et al., 1979; McGregor, 1981; Cragin et al., 1990). Limited reporting (abstracts, secondary sources) precluded critical review of these studies.

Quantitative data were not presented in a report of an assay in which SCEs were not observed in bone marrow of mice (Paika et al., 1981).

8.7   Reproductive toxicity

8.7.1   Effects on fertility

Effects on organ weights or histopathological effects in the reproductive organs have not been observed in medium-term or long-term studies in rats or mice following inhalation or oral exposure (Becci et al., 1983; Craig et al., 1984; Kennedy & Sherman, 1986; NTP, 1992a; Malley et al., 1994). In several of these bioassays, additional reproductive end-points were examined. These included sperm density, motility, or count and length of diestrus in rats and mice exposed for 13 weeks to concentrations up to 800 ppm (2400 mg/m^3) (NTP, 1992a) and semen volume and sperm motility, morphology, or count in a limited number of monkeys exposed to 500 ppm (1500 mg/m^3) (Hurtt et al., 1992). In none of these investigations, however, were there adverse effects on reproductive parameters at concentrations or doses less than those at which hepatic effects were observed; indeed, the only effect reported was prolonged diestrus in female rats exposed to 800 ppm (2400 mg/m^3) for 13 weeks (NTP, 1992a).

Few studies were identified in which the protocols were designed specifically to address reproductive toxicity. In a study reported as abstracts (Lewis et al., 1979; Cragin et al., 1990), exposure of male Sprague-Dawley rats to 30 or 300 ppm (90 or 900 mg/m^3) for 6 h/day for 5 days did not result in histopathological changes in reproductive organs after 6 weeks. Pairing of the exposed males with unexposed females for 6 weeks after exposure resulted in a reduced number of viable fetuses per dam in the low-dose group only.

In a multi-generation study in Swiss mice, DMF was administered in the drinking-water at concentrations of 0, 1000, 4000, or 7000 mg/litre (NTP, 1992b; Fail et al., 1998). Litters from F0 animals were sacrificed immediately. At week 16, pairs were separated and the final litters reared to postnatal day 21, then entered into an F1 fertility assessment. A crossover mating trial was also carried out with the F0 mice. The lowest level of exposure (1000 mg/litre; average 219 mg/kg body weight per day) was designated by the authors as the maximum tolerated dose (LOEL) for the F0 mice, based upon increased relative liver weight in males and females and increased relative kidney and adrenal weight in females. Reproductive effects in F0 mice included reduced fertility and fecundity at 4000 and 7000 mg/litre. The crossover trial identified females as the affected sex. Following F1 mating, both F2 litter size and live pup weight were reduced at all doses. At necropsy, the body weight of F1 males and females was reduced at the two highest doses, and both absolute and relative liver weights were increased at all doses. The authors concluded that both reproductive and developmental toxicity occurred at the two highest doses (4000 and 7000 mg/litre) in the F0 mice and at all dose levels ($1000 mg/litre) in the F1 mice.

No abnormalities were observed in sperm in an adequate single-injection study in mice, for which few details were presented (Antoine et al., 1983). Although negative results were reported in other assays in mice, quantitative data were not presented (Topham, 1980, 1981) or only a secondary source was available (McGregor, 1981).

8.7.2   Developmental toxicity

The database on developmental toxicity is more extensive, with numerous studies having been conducted in various species by the inhalation, oral, and dermal routes. Emphasis here is on the most recent studies for which protocols and reporting are most extensive.

In studies in which DMF has been administered by inhalation or ingestion, it has been, at most, weakly teratogenic, with malformations being observed only at high doses that were maternally toxic (450 ppm [1350 mg/m^3] by inhalation in rabbits; 503 mg/kg body weight per day following ingestion in rats), based on consideration of maternal body weight and signs of overt toxicity (Hellwig et al., 1991). In general, DMF has induced primarily fetotoxic effects most often at maternally toxic concentrations or doses (100 mg/kg body weight per day by stomach tube in rats) (Saillenfait et al., 1997) but occasionally in the absence of maternal toxicity, based on determination of body weight gain and overt signs. For example, Lewis et al. (1992) reported maternal weight gain in Crl:CD rats at 300 ppm (900

mg/m^3) (maternal LOEC), but not at 30 ppm (90 mg/m^3), at which concentration there was a slight but significant reduction in fetal weight. The mean fetal weights of control, low-dose, and high-dose groups were 5.5 ± 0.2, 5.5 ± 0.4, and 5.3 ± 0.2 g, respectively ($P < 0.05$ for both low- and high-dose groups).

The pattern of results of studies by the dermal route was similar, with malformations being observed in rats only at doses that were maternally toxic based on examination of weight gain and overt signs of toxicity only (944 mg/kg body weight per day in rats; 400 mg/kg body weight per day in rabbits; 944 mg/kg body weight per day in mice) (Hellwig et al., 1991). In one of the relatively recent investigations by other authors (Hansen & Meyer, 1990), fetotoxic effects (delayed ossification) only were observed at doses (945 mg/kg body weight per day) at which there were no effects on maternal weight gain and no overt signs of maternal toxicity.

Klug et al. (1998) carried out a mouse limb bud assay with DMF, HMMF, NMF, SMG (a synthesis product of glutathione and methyl isocyanate), *S*-(*N*-methyl-carbamoyl)cysteine (SMC), *N*-acetoximethyl-*N*-methylformamide (AMMF), AMCC, L-cysteine, and glutathione. There were no signs of adverse developmental effects caused by DMF, NMF, HMMF, AMMF, L-cysteine, or glutathione. However, a pronounced impact upon growth and development was observed for AMCC, SMC, and SMG (metabolites resulting from the glutathione binding pathway). The authors concluded that the developmental toxicity of DMF in different species is related to the magnitude of glutathione binding.

8.8   Neurological effects

In male Wistar rats exposed to 0, 7, 35, or 65 mg DMF/kg body weight per day in drinking-water for either 2 or 7 weeks, glial cell fractions were isolated from the left cerebral hemisphere and assayed for activity of acid proteinase and 2',3'-cyclic nucleotide 3'-phosphohydrolase (Savolainen, 1981). The right cerebral hemisphere was assayed for RNA, glutathione, and activities of succinate dehydrogenase and azoreductase. After 2 weeks, there was a dose-related increase in activity of 2',3'-cyclic nucleotide 3'-phosphohydrolase, which was significant ($P < 0.001$) at all levels of exposure. After 7 weeks of exposure to 0, 8, 39, or 75 mg/kg body weight per day, the intake of drinking-water was significantly reduced at all levels of exposure. There was also a significant reduction in activity of azoreductase and succinate dehydrogenase (uneven dose–response).

9. EFFECTS ON HUMANS

Consistent with the results of studies in experimental animals, available data from case reports and cross-sectional studies in occupationally exposed populations consistently indicate that the liver is the target organ for the toxicity of DMF in humans. The profile of effects is consistent with that observed in experimental animals, with related symptoms, increases in serum hepatic enzymes, and histopathological effects being reported.

9.1   Effects on the liver

Case reports in workers acutely exposed to DMF confirm that the liver is the target organ, with hepatic effects and associated disorders of the digestive system being reported. Symptoms include abdominal pain, anorexia, incoordination, and jaundice, as well as nausea, vomiting, and diarrhoea; nasal and skin irritation have also been reported (Tolot et al., 1968; Potter, 1973; Chary, 1974; Chivers, 1978; Guirguis, 1981; Paoletti et al., 1982a, 1982b; Riachi et al., 1993; Drouet D'Aubigny et al., 1998; Huang et al., 1998). Changes in both liver function (Weiss, 1971; Potter, 1973; Guirguis, 1981; Paoletti et al., 1982b; Riachi et al., 1993; Drouet D'Aubigny et al., 1998) and morphology (Tolot et al., 1968; Riachi et al., 1993) have also been observed. In one of the few reports where there was some indication of magnitude of exposure, hepatic impairment (marked increases in serum levels of ALT, aspartate aminotransferase [AST], AP, and bilirubin, together with fulminant hepatitis and jaundice) was reported in a woman who ingested about 0.6 g DMF/kg body weight (in a formulation containing other ingredients) in a suicide attempt (Nicolas et al., 1990). Similarly, clinical measurements were carried out in a patient who intravenously injected (presumably) 50 ml of a veterinary euthanasia drug containing DMF as a solvent (Buylaert et al., 1996). Serum AST and ALT increased, there was a transient rise in total serum bilirubin, and prothrombin time decreased. AP levels remained within the normal range.

Alcohol intolerance, characterized by flushing of the face, dizziness, nausea, and tightness of the chest, has been widely reported among DMF-exposed workers (Lyle, 1979; Lyle et al., 1979; Lauwerys et al., 1980; Yonemoto & Suzuki, 1980; Paoletti & Iannaccone, 1982; Paoletti et al., 1982a; Tomasini et al., 1983; Cirla et al., 1984; Redlich et al., 1988, 1990; Wang et al., 1989, 1991; Cai et al., 1992; Fiorita et al., 1997; Wrbitzky, 1999). While it is difficult to establish with

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Table 4: Effects of DMF exposure on hepatic function in humans.[a]

Concentration[b]	Effect on liver enzymes	Exposed population	Confounders	Reference
<10–60 ppm; random area sampling	increase	183 workers	some workers were also exposed to solvents	Wang et al. (1989, 1991)
10–42 ppm; area monitoring	increase	13 workers	few details reported	Yang et al. (1994)
1–27 ppm	no effect	27 workers		Paoletti & Iannaconne (1982)
5–20 ppm	increase (significance not reported)	13 workers	exposure to solvents	Tomasini et al. (1983)
3–20 ppm (TWA, 7 ppm); personal sampling	significant increase	100 workers		Cirla et al. (1984)
0.3–15.5 ppm (usually <10 ppm); static area sampling	no effect	22 workers		Lauwerys et al. (1980)
1–5 ppm; personal and area sampling	no effect	6 workers		Yonemoto & Suzuki (1980)
4–8 ppm (mean 6 ppm); sampling not specified	no effect	28 workers		Catenacci et al. (1984)
0.2–8 ppm; area sampling	increase (significance not reported)	26 workers	concomitant exposure to acrylonitrile	Major et al. (1998)
7 ppm; area sampling at different workplaces	significant increase	75 workers		Fiorito et al. (1997)
0.1–7 ppm; personal sampling	no effect	207 workers	some workers were also exposed to toluene	Cai et al. (1992)
up to 2.3 ppm; personal sampling	no effect	126 workers		Wrbitzky & Angerer (1998); Wrbitzky (1999)

[a] See text for more detailed descriptions of highlighted studies.

[b] 1 ppm = 3 mg/m^3.

any certainty a lowest concentration at which increases in these subjective symptoms first appear, they have been associated with mean or median levels of 10 ppm (30 mg/m^3) (Lauwerys et al., 1980; Yonemoto & Suzuki, 1980; Cai et al., 1992; Fiorito et al., 1997); in a recent study, some workers reported symptoms upon exposure to concentrations for which the median value was as low as 1.2 ppm (3.6 mg/m^3) (Wrbitzky, 1999).

Levels of serum hepatic enzymes in populations occupationally exposed to DMF have been determined in several cross-sectional studies. A brief overview of the information on exposure–response derived from these studies is summarized in Table 4.

While there have been considerable variations in the size of study populations, magnitude and duration of exposure, extent of exposure to other substances, and adequacy of reporting in these investigations, there is a consistent pattern of increase in serum enzymes in workers with relatively higher exposures in these investigations, some of which included individual monitoring. In summary, the results concerning exposure–response are consistent across studies, with increases in serum hepatic enzymes not being observed at concentrations

in the range of 1–6 ppm (3–18 mg/m^3). At higher levels of exposure (>7 ppm [>21 mg/m^3]), increased serum levels of hepatic enzymes have been observed consistently.

There were three studies identified (highlighted in Table 4) for which TWA exposures were presented and which can serve, therefore, as the basis for at least crude estimates of exposure–response. These are described in more detail here. It should be noted, though, that the monitored levels in these studies do not take into account potential additional dermal exposure.

In a carefully conducted investigation of liver function in 75 workers in a synthetic leather factory, geometric mean levels of DMF in the air based on 8-h area sampling in various working locations were approximately 20 mg/m^3 (~7 ppm) (range 2–40 mg/m^3) (Fiorito et al., 1997). It was reported that the study subjects worked in a factory that produces synthetic leather using polyurethane resin, pigments, and large amounts of DMF (about 14 tonnes/day), where skin contact with liquid DMF was also possible. The mean duration of employment was 3.8 years. The control group consisted of 75 unexposed workers similar in age, sex, social status, and residence. Confounding by

alcohol consumption and pre-existing liver disease was minimized through selection criteria for study subjects. Analysis of paired enzymes was also conducted. All workers underwent a complete physical examination, with liver function tests for serum AST, ALT, ($-glutamyl transpeptidase (($-GT), AP, bile acids (BA), bilirubin, serum cholesterol and triglycerides, and markers for hepatitis A, B, and C. Gastrointestinal symptoms (stomach pain, nausea, appetite loss) were reported by 50% of the DMF-exposed workers, and 40% had symptoms such as face flushing, palpitation, headache, dizziness, or tremors following alcohol consumption. (Many avoided alcohol as a result.) Mean serum ALT (28.8 vs. 21.9 IU/litre), AST (26.5 vs. 21.1 IU/litre), ($-GT (29.5 vs. 14.2 IU/litre), and AP (75.7 vs. 60.8 IU/litre) were significantly higher in 12 of 75 workers in the exposed group ($P < 0.001$); 17/75 (23%) had abnormal liver function, compared with only 4% of controls. Multivariate analyses confirmed that ALT, AST, and ($-GT were significantly correlated with cumulative DMF exposure. The analyses controlled for factors such as body mass index, alcohol intake, serum cholesterol, and hepatitis markers, which did not explain the observed effects.

Catenacci et al. (1984) investigated liver function (serum glutamate–oxalate transaminase [SGOT], serum glutamate–pyruvate transaminase [SGPT], ($-GT, and AP) in workers employed for at least 5 years in an acrylic fibre plant; no mention was made of exposure to other solvents. The first group of 28 subjects worked in the spinning department, where DMF exposure (8-h TWA) ranged from 12 to 25 mg/m^3, with a mean of 18 mg/m^3 (4–8 ppm, mean 6 ppm). The second group consisted of 26 subjects exposed, in the polymer department, to DMF at (8-h TWA) 1.8–5 mg/m^3, with a mean of 3 mg/m^3 (0.6–1.8 ppm, mean 1 ppm). A control group consisted of 54 subjects matched for age, smoking/alcohol consumption, and history of liver disease, who had never been occupationally exposed to solvents. The data on which the estimated TWA exposures were based were not reported. Mean serum values for SGOT (20.74, 21.06, and 20.17 mU/ml for 6 ppm, 1 ppm, and control groups, respectively), SGPT (19.76, 21.26, and 26.09 mU/ml for 6 ppm, 1 ppm, and control groups, respectively), ($-GT (36.37, 28.34, and 40.76 mU/ml for 6 ppm, 1 ppm, and control groups, respectively), and AP (154.42, 150.35, and 153.07 mU/ml for 6 ppm, 1 ppm, and control groups, respectively) did not differ among the three groups and were within the normal ranges. Few additional details were presented in the published account of this study.

Cirla et al. (1984) carried out a clinical evaluation of 100 workers in synthetic polyurethane leather produc

tion exposed to a mean TWA concentration (determined by personal sampling) of 22 mg/m^3 (range 8–58 mg/m^3) (mean TWA 7 ppm; range 3–19 ppm). The mean exposure period was 5 years (range 1–15 years). The workers were also exposed to small (but unspecified) quantities of toluene, methyl ethyl ketone (MEK), ethyl acetate, and isopropyl and isobutyl alcohol. Study subjects were selected to minimize large variations in exposure; those with histories of possible accidental exposures were also excluded. The referent group was 100 workers at the same or similar factories, without exposure to any solvents or toxic metals, matched by sex, age group, alcohol history, smoking habits, coffee intake, socio-economic status, residence, and dietary customs. Clinical evaluation was carried out and a laboratory assessment was performed for blood cell counts and serum AP, AST, ALT, and ($-GT. Serum ($-GT was abnormally high in 25/100 exposed and only 10/100 referents ($P < 0.01$). Higher prevalences in the exposed group for abnormally high serum levels of AST (9 vs. 3) and ALT (12 vs. 8) were not statistically significant. AP values were normal in all subjects. When subjects who had not modified their alcohol consumption upon working with DMF were considered, the effect was still evident. Several symptoms, including headache, dyspepsia, and digestive impairment, characteristic of effects on the liver were also associated with exposure to DMF.

Histopathological changes in liver have also been reported in occupationally exposed workers, although quantitative data on levels of exposure are not well documented. Tomasini et al. (1983) reported hepatic pain and palpable liver in 4 of 13 workers exposed to 5–20 ppm (15–60 mg/m^3) DMF (and other solvents), ranging from a few weeks to 4 years. Redlich et al. (1990) carried out biopsies of liver from workers heavily exposed to DMF (and other solvents; quantitative data not reported). Workers exposed for less than 3 months had hepatocellular necrosis, enlarged Kupffer cells, microvesicular steatosis, complex lysosomes, and pleomorphic mitochondria. The livers of workers exposed for longer terms (14–120 months) had fatty changes with occasional lipogranuloma.

9.2 Cardiac effects

Excess mortality from ischaemic heart disease in DMF-exposed workers in a US acrylonitrile fibre plant was observed in a historical cohort study (Chen et al., 1988b). Between 1950 and 1982, there were 62 deaths due to ischaemic heart disease (40.3 expected from company rates; $P < 0.01$). The increase was not significant in comparison with the state (South Carolina) rates. A similar observation was made for a second group of 1329 employees at the plant who were potentially exposed to

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both DMF and acrylonitrile (65 deaths observed, 48.3 expected from company rates; $P < 0.05$). However, the observed number of deaths was not significantly higher than that which would be expected from either state or national rates, possibly due to a "healthy worker effect." Lifestyle factors such as alcohol and tobacco consumption were suggested to be more likely causes than exposure to DMF, although the specific basis for this contention was not specified (Chen et al., 1988b). The authors noted that South Carolina has a higher ischaemic heart disease mortality rate than the USA.

No convincing evidence of adverse effects on cardiac function was seen in a limited study in which electrocardiographic (ECG) monitoring was carried out on workers at a small synthetic leather plant where DMF was used. Monitoring of eight workers over a workshift revealed possible mild effects (isolated ventricular premature beats after 2 h of work, without "pathological alteration" of the ECG) in one worker (Taccola et al., 1981). In a brief report, ECG changes in workers exposed to DMF were reported (<3 ppm [<9 mg/m^3], with peaks up to 1500 ppm [4500 mg/m^3], plus skin exposure), but little detail was provided (Kang-De & Hui-Lan, 1981).

Cardiac disturbances, including tachycardia and palpitations, have occasionally been observed in cross-sectional studies of DMF-exposed workers (Lyle, 1979; Lyle et al., 1979; Kang-De & Hui-Lan, 1981; Cirla et al., 1984; Fiorito et al., 1997). Sometimes, the palpitations followed alcohol ingestion (Lyle, 1979; Lyle et al., 1979; Fiorito et al., 1997).

9.3   Cancer

Data on the incidence or mortality of cancer associated with exposure to DMF are limited to case reports of testicular tumours and single well conducted and reported cohort and case–control studies of occupationally exposed populations (Chen et al., 1988a; Walrath et al., 1989). In the cohort study of 3859 actively employed workers with potential exposure to DMF and to DMF and acrylonitrile in an acrylonitrile fibre production facility, the incidences of cancer of the buccal cavity/pharynx, lung, prostate, stomach, nervous system, and bladder were considered in relation to level of and, for some tumours, duration of exposure and were compared with company and national rates. Level of exposure was classified as low (approximately <10 ppm (<30 mg/m^3)), moderate (sometimes above 10 ppm [30 mg/m^3]), or high, although quantitative data were not reported (Chen et al., 1988a). In an additional case–control study, cancers of the buccal cavity/pharynx ($n = 39$), liver ($n = 6$), prostate ($n = 43$), and testis ($n = 11$) and

malignant melanoma of the skin ($n = 39$) were examined in approximately 8700 workers from four plants, which included a DMF production plant, two acrylic fibre plants that used DMF as a spinning solvent, and a plant using the chemical as a solvent for inks (Walrath et al., 1989).

Three cases of testicular germ cell tumours that occurred during 1981–1983 among 153 white men who repaired the exterior surfaces and electrical components of F4 Phantom jets in the USA were reported by Ducatman et al. (1986), which led to surveys of two other repair shops at different locations, one in which F4 Phantom jets were repaired and one where other types of aircraft were repaired. Four of 680 workers in the F4 Phantom shop had testicular germ cell cancers (approximately one expected) diagnosed during 1970–1983. No cases were reported in the other facility. All seven men had long histories in aircraft repair; although there were many common exposures to solvents in the three facilities, the only one identified as unique to the F4 Phantom jet aircraft repair facilities was to a solvent mixture containing 80% DMF (20% unspecified). Three of the cases had been exposed to this mixture with certainty, and three had probably been exposed. Of the seven cases, five were seminomas and two were embryonal cell carcinomas.

Levin et al. (1987) and Frumin et al. (1989) reported three cases of embryonal cell carcinoma of the testis in workers at one leather tannery in the USA, where it was reported that DMF as well as a wide range of dyes and solvents were used, including such testicular toxins as 2-ethoxyethanol and 2-ethoxyethanol acetate. The latency period ranged from 8 to 14 years. No additional cancers were reported in a screening effort undertaken to identify additional testicular cancers in 51 of the 83 workers at the leather tannery where the three cases were reported (Calvert et al., 1990).

In an investigation of cancer incidence at a plant producing acrylonitrile fibres, compared with company and national rates, there was no increase in the incidence of testicular cancer in 2530 actively employed workers exposed to DMF only. When the data from this cohort were grouped with data from 1329 workers exposed to both DMF and acrylonitrile, there was only one case of testicular cancer, versus 1.7 expected (confidence interval [CI] not reported) (Chen et al., 1988a).

There was no increase in cancer of testis (odds ratio = 0.91; 95% CI = 0.1–8.6; observed number of cases = 11) in the case–control study described above in which the cases were drawn from a population of

approximately 8700 workers involved in production or use of DMF at four plants (Walrath et al., 1989, 1990). For each case, two controls were selected, matched for age, sex, payroll class, and plant. Potential exposure to DMF was classified as low or moderate based on job title/work area combinations and monitoring data.

Chen et al. (1988a) observed a significant increase in prostate cancer (10 observed vs. 5.1 expected from company rates and 5.2 expected from national rates; $P < 0.10$ for both comparisons) in the 3859 workers exposed either to DMF or to both DMF and acrylonitrile. However, when only DMF-exposed workers (2530) were considered, the standardized incidence rate (SIR) (4 observed vs. 2.4 expected from company rates) was not significant. The odds ratio for prostate cancer in the case–control study of the 8700 DMF-exposed workers from four plants was not significantly elevated (1.48; 95% CI = 0.59–3.74; 43 cases) (Walrath et al., 1989, 1990). When analyses were carried out separately for each of the four plants, an increased incidence was observed only at one plant, where the exposure to DMF was lower and the number of cases was fewer than at the other plants. Adjustment for assumed latency period did not alter the odds ratio. There was no relationship with duration of exposure.

Chen et al. (1988a) also reported a significant increase of cancer of the buccal cavity/pharynx (9 observed vs. 1.6 expected from company rates; $P < 0.10$) in the 2530 DMF-exposed workers (confidence intervals not reported). When combined with data from 1329 workers exposed to both DMF and acrylonitrile, the increase (11 observed) was significant when compared with the company rate (3.2 expected; $P < 0.01$), but not compared with the national rate (6.6 expected). There was no relation to either level or duration of exposure. All cases were heavy, long-term smokers. There was no increase in risk of cancer of the buccal cavity/pharynx in the case–control study of workers at the four plants mentioned above (odds ratio = 0.89; 90% CI = 0.35–2.29; 39 cases) (Walrath et al., 1989, 1990).

9.4 Genotoxicity

Seven studies were identified in which the genotoxicity of DMF in humans has been examined. Four of these studies were critically reviewed by IARC (1999) and were described therein as follows.

Berger et al. (1985) reported that the prevalence of CAs was higher in the blood lymphocytes of 20 workers exposed to DMF, NMF, and dimethylamine than in 18 unexposed workers at the same factory (1.4% vs. 0.4%;

statistical significance not provided). The mean concentrations 1 year prior to blood sampling were 12.3 mg/m^3 for DMF, 5.3 mg/m^3 for NMF, and 0.63 mg/m^3 for dimethylamine. However, the control group had an unusually low level of chromosome breaks. The IARC Working Group noted that the possible effect of smoking was not addressed.

A higher incidence of CAs was observed in the lymphocytes of about 40 workers exposed to DMF than in an unspecified control group (2.74–3.82% vs. 1.10–1.61%; $P < 0.05$). The range of exposure to DMF was 150–180 mg/m^3. Workers were also exposed to trace amounts of MEK, butyl acetate, toluene, cyclohexanone, and xylene. After technological improvements designed to reduce DMF exposure levels (range 35–50 mg/m^3), the frequency of aberrant cells decreased to 1.49–1.59% (Koudela & Spazier, 1981).

Although Sram et al. (1985) reported in an abstract that there was no evidence of increased frequency of CA in peripheral lymphocytes in workers exposed to DMF, no details were provided.

Seiji et al. (1992) reported that the mean SCE rate was higher in the blood cells of 22 women exposed to three concentrations of DMF (0.3–5.8 ppm [0.9–17.4 mg/m^3]) in a leather production factory than in 22 unexposed controls from the same factory, matched by sex, age, and residence. None of the women smoked tobacco or drank alcohol. The incidence of SCEs was significantly increased in a dose-related manner in the mid- and high-exposure groups.

Based on review of these studies, IARC (1999) concluded that "The positive data for cytogenetic damage in humans occupationally exposed to it are not very convincing."

Three relevant reports, including one for which only an abstract was identified in which few details were provided (Haber et al., 1990), were identified in addition to those reviewed by IARC (1999). The two investigations for which reporting was adequate are described here.

Major et al. (1998) reported that for workers with 3–10 years of occupational exposure to undefined levels of DMF and/or acrylonitrile, the prevalence of peripheral lymphocytes with CAs was increased compared with unexposed controls (see below). After a further 7 months of exposure (to DMF at 0.2–8 ppm [0.6–24 mg/m^3] and to acrylonitrile at 0–17.6 mg/m^3), the incidence in the exposed group increased to 5.1% but did not increase further up to 20 months. The incidence of SCEs was also

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higher than control values at the start of the 20-month study and remained higher at 7 and 20 months. The UDS level was similar to that in controls when the study started, but had increased in the exposed group by month 7. In addition to concomitant exposure to acrylonitrile, current smoking was also a confounding factor, with CA and SCE yields being significantly higher in exposed smokers than in exposed non-smokers. Nevertheless, CA yields at 7 months were significantly higher in exposed non-smokers than in control non-smokers and in exposed smokers than in control smokers.

Cheng et al. (1999) measured SCE frequency in peripheral lymphocytes of workers at a resin synthesis plant. Nine workers had low exposure (median 5.2 ppm [15.6 mg/m^3]; range 0.9–5.3 ppm [2.7–15.9 mg/m^3]), and 20 workers had high exposure (median 24.8 ppm (74.4 mg/m^3); range 11.4–83.3 ppm [34.2–249.9 mg/m^3]). There were no differences between the two groups; there was no additional control population.

Results of studies on genotoxicity conducted since the IARC evaluation have not contributed materially to the database, which was considered by IARC (1999) not to provide convincing evidence. Certainly, the results, when taken as a whole, are inconsistent and not readily explained by variations in exposure.

10. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

DMF has been the focus of several toxicity studies conducted on a range of species. The most sensitive end-points for terrestrial and aquatic organisms are presented below and are summarized in Table 5.

10.1   Aquatic environment

A number of studies are available for a range of taxa, including protozoa, blue-green algae, diatoms, green algae, macrophytes, molluscs, oligochaetes, crustaceans, insect larvae, and fish.

For four species of fish, EC_{50} and LC_{50} values ranged from approximately 7100 to 12 000 mg/litre (Batchelder, 1976; Johnson & Finley, 1980; Call et al., 1983; Poirier et al., 1986; Groth et al., 1994). The most sensitive fish species appears to be the bluegill (*Lepomis macrochirus*), with an LC_{50} of 7100–7500 mg/litre.

Aquatic invertebrates tested include the water flea (*Daphnia magna*) and various species of insect larvae.

The water flea appears to be the most sensitive invertebrate, with a NOEL of 1140 mg/litre. Acute end-points (EC_{50} and LC_{50}) for *Daphnia magna* range from 12 400 to 15 700 mg/litre, whereas chronic studies provide end-points for mortality between 1140 and 3721 mg/litre (Call et al., 1983; Leblanc & Surprenant, 1983; Adams & Heidolph, 1985; Poirier et al., 1986; Ziegenfuss et al., 1986; Sebaugh et al., 1991). The 48-h LC_{50}s obtained for various species of insect larvae were much higher and ranged from 33 500 to 36 200 mg/litre (Call et al., 1983; Poirier et al., 1986; Ziegenfuss et al., 1986).

The most sensitive alga appears to be *Selenastrum capricornutum*, with a 14-day NOEC value for growth inhibition of 480 mg/litre (Hughes & Vilkas, 1983). Results for two other green algae range from 8900 to 10 000 mg/litre (Stratton & Smith, 1988; El Jay, 1996). Peterson et al. (1997) obtained an IC_{25} for growth inhibition of 6200 mg/litre for the diatom *Nitzschia* sp. In the same study, blue-green algae appeared to be the least sensitive, with IC_{25}s for growth inhibition ranging from 7000 to 15 100 mg/litre for three tested species (Peterson et al., 1997), a finding that differs from earlier data (Stratton, 1987). Because of the high degree of quality assurance/quality control associated with the Peterson et al. (1997) study, these data are considered as definitive levels of toxicity to blue-green algae.

Rajini et al. (1989) measured the lethal response of the ciliated protozoan *Paramecium caudatum* to acute (10-min and 4-h) exposures to DMF. The 4-h LC_{50} was found to be 20 465 mg/litre. A recent paper reports EC_{50}s of 8190–9870 mg/litre for deformations and LC_{50}s of 19 700–31 700 mg/litre for the protozoan *Spirostomum ambiguum* (Nalecz-Jawecki & Sawicki, 1999).

Marine organisms tested include the bacteria *Vibrio fischeri*, the common shrimp (*Crangon crangon*), and a fish, the winter flounder (*Pleuronectes americanus*). For the decrease in luminescence in *Vibrio fischeri*, the 5-min EC_{50} value of 20 000 mg/litre (Curtis et al., 1982) is in the same order of magnitude as the values (13 260–14 830 mg/litre) obtained by Harwood[1] with a 15-min exposure. IC_{25} values calculated by Harwood[1] with the same data set range from 5830 to 6730 mg/litre.

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[1] Personal communications from M. Harwood, Environment Canada, to A. Chevrier, Environment Canada, dated 2 and 5 December 1997.

N,N-Dimethylformamide

Table 5: Toxicity of DMF to various organisms.

Test species	Latin name	End-point	Range	References
Bacteria	*Vibrio fischeri*	5-min EC_{50} light production	20 000 mg/litre	Curtis et al. (1982)
Bacteria	*Vibrio fischeri*	15-min IC_{50} light inhibition 15-min IC_{25} light inhibition	13 260–14 830 mg/litre 5830–6730 mg/litre	Harwood[a]
Protozoan	*Paramecium caudatum*	4-h LC_{50} mortality	20 465 mg/litre	Rajini et al. (1989)
Protozoan	*Spirostomum ambiguum*	24-h EC_{50} deformations 24-h LC_{50} mortality 48-h EC_{50} deformations 48-h LC_{50} mortality	9870 mg/litre 31 700 mg/litre 8190 mg/litre 19 700 mg/litre	Nalecz-Jawecki & Sawicki (1999)
Blue-green algae	*Nostoc* sp.	10- to 14-day EC_{50} growth inhibition test	<480 mg/litre	Stratton (1987)
Blue-green algae	*Anabaena* sp.	10- to 14-day EC_{50} growth inhibition test	<480 mg/litre	Stratton (1987)
Blue-green algae	*Anabaena cylindrica*	10- to 14-day EC_{50} growth inhibition test	<480 mg/litre	Stratton (1987)
Blue-green algae	*Anabaena variabilis*	10- to 14-day EC_{50} growth inhibition test	<480 mg/litre	Stratton (1987)
Blue-green algae	*Anabaena inaequalis*	10- to 14-day EC_{50} growth inhibition test	5700 mg/litre	Stratton (1987)
Blue-green algae	*Anabaena flos-aquae*	48-h IC_{25} growth inhibition	15 100 mg/litre	Peterson et al. (1997)
Blue-green algae	*Microcystis aeruginosa*	48-h IC_{25} growth inhibition	7000 mg/litre	Peterson et al. (1997)
Blue-green algae	*Oscillatoria* sp.	48-h IC_{25} growth inhibition	10 400 mg/litre	Peterson et al. (1997)
Diatom	*Nitzschia* sp.	48-h IC_{25} growth inhibition	6200 mg/litre	Peterson et al (1997)
Green algae	*Selenastrum capricornutum*	48-h IC_{25} growth inhibition	7700 mg/litre	Peterson et al. (1997)
Green algae	*Selenastrum capricornutum*	72-h IC_{25} growth as cell numbers	3420–6280 mg/litre	Harwood[a]
Green algae	*Selenastrum capricornutum*	growth at day 4	inhibition at 5000 mg/litre	El Jay (1996)
Green algae	*Selenastrum capricornutum*	growth inhibition NOEC	480 mg/litre	Hughes & Vilkas (1983)
Green algae	*Selenastrum capricornutum*	growth at day 4	stimulation at 1000 mg/litre	El Jay (1996)
Green algae	*Chlorella vulgaris*	growth at day 4	inhibition at 10 000 mg/litre	El Jay (1996)
Green algae	*Chlorella vulgaris*	growth at day 4	stimulation at 1000 mg/litre	El Jay (1996)
Green algae	*Chlorella pyrenoidosa*	10- to 14-day EC_{50} reduction in growth	8900 mg/litre	Stratton & Smith (1988)
Duckweed	*Lemna minor*	7-day IC_{25} growth inhibition	4900 mg/litre	Peterson et al. (1997)
Water flea	*Daphnia magna*	acute 48-h EC_{50} immobilization	14 500 mg/litre	Poirier et al. (1986)
Water flea	*Daphnia magna*	acute 48-h EC_{50} survival and mortality	15 700 mg/litre	Adams & Heidolph (1985)
Water flea	*Daphnia magna*	acute 48-h LC_{50} mortality	14 400 mg/litre	Ziegenfuss et al. (1986)
Water flea	*Daphnia magna*	acute 48-h LC_{50} mortality	14 530 mg/litre	Call et al. (1983)
Water flea	*Daphnia magna*	acute 48-h EC_{50} immobilization	13 100 mg/litre	Sebaugh et al. (1991)

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Table 5 (contd).

Test species	Latin name	End-point	Range	References
Water flea	*Daphnia magna*	chronic 21-day EC_{50} survival and mortality	3721 mg/litre	Adams & Heidolph (1985)
Water flea	*Daphnia magna*	chronic 21-day NOEL/LOEC survival and mortality	1500–3000 mg/litre	Adams & Heidolph (1985)
Water flea	*Daphnia magna*	chronic 28-day NOEL survival and mortality	1140 mg/litre	Leblanc & Surprenant (1983)
Water flea	*Daphnia magna*	acute 48-h EC_{50} survival and mortality	12 400 mg/litre	Leblanc & Surprenant (1983)
Insect larvae	*Paratanytarsus parthenogeneticus*	48-h EC_{50}	36 200 mg/litre	Poirier et al. (1986)
Insect larvae	*Tanytarsus dissimilis*	48-h LC_{50}	36 000 mg/litre	Call et al. (1983)
Insect larvae	*Chironomus tentans*	acute 48-h LC_{50} mortality	33 500 mg/litre	Ziegenfuss et al. (1986)
Shrimp	*Crangon crangon*	LC_{50}	>100 mg/litre	Portmann & Wilson (1971)
Rainbow trout	*Oncorhynchus mykiss*	acute 96-h LC_{50} mortality	9800–12 000 mg/litre	Johnson & Finley (1980); Call et al. (1983); Poirier et al. (1986)
Winter flounder	*Pleuronectes americanus*	inhibition of the enzyme activity in intestinal mucosae	50 000 mg/litre	Janicki & Kinter (1971)
Zebrafish	*Brachydanio rerio*	acute 96-h LC_{50} mortality	8840 mg/litre	Groth et al. (1994)
Fathead minnow	*Pimephales promelas*	acute 96-h LC_{50} mortality	9080–11 400 mg/litre	Batchelder (1976); Call et al. (1983); Poirier et al. (1986)
Bluegill	*Lepomis macrochirus*	acute 96-h LC_{50} mortality	7100–7500 mg/litre	Call et al. (1983); Poirier et al. (1986)
Soil fungi	*Sclerotinia homeocarpa*	EC_{50} inhibition of fungal growth, compared with a control growth of 50–70 mm	4840 mg/litre	Stratton (1985)
Soil fungi	*Pythium ultimum*	EC_{50} inhibition of fungal growth, compared with a control growth of 50–70 mm	10 250 mg/litre	Stratton (1985)
Soil fungi	*Pestalotia* sp.	EC_{50} inhibition of fungal growth, compared with a control growth of 50–70 mm	5970 mg/litre	Stratton (1985)
Wheat and bean seeds		inhibition of germination	50 000 mg/litre	Szabo (1972)
Rat		2-year inhalational NOEL, 6 h/day, 5 days/week exposure changes in body weight and clinical chemistry parameters	75 mg/m^3	Malley et al. (1994)

[a] Personal communications from M. Harwood, Environment Canada, to A. Chevrier, Environment Canada, dated 2 and 5 December 1997.

Portmann & Wilson (1971) reported an LC_{50} of >100 mg/litre for *Crangon crangon*.

10.2   Terrestrial environment

There is little information available on the toxicity of DMF to terrestrial vascular plants. Szabo (1972) reported that DMF did not inhibit germination of wheat and bean seeds at 1% (approximately 10 000 mg/litre), but did at 5% (approximately 50 000 mg/litre); however, little methodological information is provided with which to assess the quality of the data. The IC_{25} of 4900 mg/litre for the duckweed (*Lemna minor*), an aquatic angiosperm, indicates that terrestrial angiosperms may not be sensitive to DMF (Peterson et al., 1997). The most sensitive organism in the terrestrial

compartment appears to be the soil fungus *Sclerotinia homeocarpa*, with an EC_{50} of 4840 mg/litre for growth inhibition (Stratton, 1985).

Although information on effects of DMF on wild-life has not been identified, a review of laboratory studies on experimental animals (WHO, 1991) concludes that acute toxicity of DMF in a variety of species is low. Only one chronic (2-year) inhalation assay was identified in recent literature (Malley et al., 1994). In that study, a LOEC of 25 ppm (75 mg/m^3) following inhalation of DMF was reported, based on changes in body weight and clinical chemistry.

11. EFFECTS EVALUATION

11.1 Evaluation of health effects

11.1.1 Hazard identification and dose–response assessment

11.1.1.1 Effects in humans

Consistent with the results of studies in experimental animals, available data from case reports and cross-sectional studies in occupationally exposed populations indicate that the liver is the target organ for the toxicity of DMF in humans. The profile of effects is consistent with that observed in experimental animals, with gastrointestinal disturbance, alcohol intolerance, increases in serum hepatic enzymes (AST, ALT, (-GT, and AP), and histopathological effects (hepatocellular necrosis, enlarged Kupffer cells, microvesicular steatosis, complex lysosomes, pleomorphic mitochondria, and fatty changes with occasional lipogranuloma) being observed. Effects observed at lowest concentrations in cross-sectional studies in occupationally exposed populations for which there is some information on dose–response are increases in serum hepatic enzymes.

Based on the limited data available, there is no convincing, consistent evidence of increased risk of cancer at any site associated with exposure to DMF in the occupational environment. Case reports of testicular cancers have not been confirmed in a cohort and case–control study. There have been no consistent increases in tumours at other sites associated with exposure to DMF.

There is also little consistent, convincing evidence of genotoxicity in populations occupationally exposed to DMF, with results of available studies of exposed workers (to DMF and other compounds) being mixed. The pattern of observations is not consistent with variations in exposure across studies. However, in view

of the positive dose–response relationship observed in the one study in which it was investigated, this area may be worthy of additional work, although available data on genotoxicity in experimental systems are overwhelmingly negative.

11.1.1.2 Effects in experimental animals

DMF has low acute toxicity and is slightly to moderately irritating to the eyes and skin, based on limited data acquired in non-standard assays. Available data are inadequate as a basis for characterization of the potential of DMF to induce sensitization. In acute and repeated-dose toxicity studies, DMF has been consistently hepatotoxic, inducing effects on the liver at lowest concentrations or doses. The profile of effects includes alterations in hepatic enzymes, increases in liver weight, progressive degenerative histopathological changes and eventually cell death, and increases in serum hepatic enzymes. Species variation in sensitivity to these effects has been observed, with the order of sensitivity being mice > rats > monkeys.

Although the database for carcinogenicity is limited to two adequately conducted bioassays in rats and mice, there have been no increases in the incidence of tumours following chronic inhalation exposure to DMF. The weight of evidence for genotoxicity is overwhelmingly negative, based on extensive investigation in *in vitro* assays, particularly for gene mutation, and a more limited database *in vivo*.

DMF has induced adverse reproductive effects only at concentrations considerably greater than those associated with adverse effects on the liver. In adequately conducted and reported primarily recent developmental studies, fetotoxic and teratogenic effects have been consistently observed only at maternally toxic concentrations or doses.

Available data are inadequate as a basis for assessment of the neurological, immunological, or skin sensitizing effects of DMF.

The following guidance is provided as a possible basis for derivation of limits of exposure and judgement of the quality of environmental media by relevant authorities.

11.1.2 Criteria for setting tolerable concentrations or guidance values

In both humans and experimental animals exposed to DMF, the target organ has been the liver, consistent with local action of a reactive intermediate in the tissue where it is primarily metabolized. Available data indicate that there are considerable variations between experimental animals and humans in the proportion of

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DMF metabolized by the putatively toxic pathway, with the resulting implication that humans may be more sensitive to the effects of DMF. Also, since there are data available to serve as the basis for at least crude characterization of exposure–response for parameters associated with hepatic toxicity in workers, the tolerable concentration (TC) is based on data on inhalation in humans, although it should be noted that these values do not account for likely additional exposure by dermal absorption. Analyses of dose–response for hepatic effects in the studies in experimental animals are presented for comparison. Since exposure in the general environment is likely to be primarily through air, emphasis in this section is on the generally more extensive database on toxicity by the inhalation route.

Effects on the liver observed at lowest concentration in cross-sectional studies in occupationally exposed populations for which there is some information on exposure–response are increases in serum hepatic enzymes. The results concerning exposure–response are consistent across studies, with increases in serum hepatic enzymes not being observed at concentrations in the range of 1–6 ppm (3–18 mg/m³). At higher levels of exposure (>7 ppm [>21 mg/m³]), increased serum levels of hepatic enzymes have been observed consistently. Cirla et al. (1984) reported significant increases in serum γ-GT in 100 workers exposed to 7 ppm (21 mg/m³). Similarly, Fiorito et al. (1997) reported significant increases in serum ALT, AST, γ-GT, and AP in workers exposed to 7 ppm (21 mg/m³).

Catenacci et al. (1984) did not observe differences between serum levels of SGOT, SGPT, and γ-GT in workers employed for more than 5 years. In view of the small number of subjects exposed to the mean TWA of 6 ppm (18 mg/m³) DMF (*n* = 28), negative results reported therein may be a function of lack of power of the study to detect a meaningful effect and are not, therefore, necessarily inconsistent with the results of Cirla et al. (1984) and Fiorito et al. (1997).

Based on the lowest-observed-adverse-effect level (LOAEL) of 7 ppm (21 mg/m³), a TC[1] has been derived as follows:

$$TC = \frac{7\,ppm}{50} \times 8/24 \times 5/7$$

$$= 0.03\ ppm\ (0.1\ mg/m^3)$$

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[1] The term "tolerable concentration" is used here in the same sense as the term "tolerable intake" as defined by IPCS (1994), i.e., "an estimate of the intake of a substance over a lifetime that is considered to be without appreciable health risk."

where:

- 7 ppm (21 mg/m³) is the LOAEL for increases in serum hepatic enzymes in workers exposed primarily to DMF, reported by Cirla et al. (1984) and Fiorito et al. (1997); it should be noted that the observed small increases in a few serum hepatic enzymes are considered to be only minimally adverse, with associated hepatic damage likely being reversible upon cessation of exposure;
- 8/24 and 5/7 are the factors to convert exposure during 8 h/day and 5 days/week, respectively, to continuous exposure;
- 50 is the uncertainty factor (×10 for intraspecies [interindividual][2] variation, including sensitive subgroups; ×5 to account primarily for less than lifetime exposure; although the TC is based on a LOAEL, observed effects are considered to be only minimally adverse).

Although not the basis of the TC developed here, there are several important observations from dose–response analyses of the results of the studies in animals (see Appendix 4). The lowest reported benchmarks for a range of hepatic effects in rats and mice following inhalation are those for histopathological lesions in the liver of both rats and mice, which are higher but in the same range as those reported to induce effects on hepatic function in the studies in workers. It should be noted, though, that, due to the nature of the effects on which they were based (increases in serum hepatic enzymes versus histological effects), the benchmarks in humans are not strictly comparable.

It is also evident that there is progression of effects from the medium-term to long-term studies, with effects being more severe following long-term exposure (although quantitative values for the lowest benchmarks for different types of lesions in the medium-term and long-term studies are similar).

11.1.3   *Sample risk characterization*

Due to the nature of use, patterns of release, and environmental fate of DMF, the focus of the human health risk characterization for indirect exposure is populations exposed through air in the vicinity of industrial point sources.

With a reported annual loading of less than 20 tonnes and generally less than 1 tonne at any location in the sample country (i.e., Canada), continuous releases of consistent magnitude likely result in long-term expo-

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[2] Available quantitative data are insufficient to replace default values for the component of this uncertainty factor with data-derived values (IPCS, 1994).

sure to small concentrations (worst-case estimate in Canada, 0.11 mg/m^3) of DMF near point sources. Because of the absence of empirical data on concentrations of DMF in air in Canada, an estimated exposure value (EEV) was calculated based on release data for the largest Canadian emitter, making several conservative assumptions.

The largest annual release reported at one location can be expressed on a daily basis (12.7 tonnes/year = 0.0348 tonnes/day or 3.48×10^7 mg/day). As a conservative estimate, it will be assumed that daily releases of DMF are contained within a cylinder having a radius of 1 km centred on the point source. Dispersion within 1 km is likely a conservative assumption for a number of reasons. First, the greatest reported emissions are occurring in a mixed industrial and agricultural area (Environment Canada, 1999b). The site is paved with asphalt; as such, wild plants and mammals will not likely be found in the immediate vicinity of the source. Finally, although the specific dispersal behaviour of DMF has not been documented near the source, results of dispersion modelling indicate that concentrations of other contaminants released to air elsewhere tend to decrease rapidly within a few kilometres of industrial point sources (e.g., Davis, 1997; Thé, 1998).

Upward movement of organic compounds generally does not exceed 100 m at night and may exceed 1000 m during the day.[1] The more conservative value of 100 m will be used as a ceiling for estimating the exposure concentrations throughout the day.

This provides a dispersal volume of 3.14×10^8 m^3 in the form of a cylinder 100 m in height and 1 km in radius. With a daily release of 3.48×10^7 mg/day, the daily increase in the concentration of DMF in air is estimated at 0.11 mg/m^3. Since ambient levels in the cylinder are likely to be lower than this daily increase of 0.11 mg/m^3, it will be used as a conservative EEV. Reaction with hydroxyl radicals will tend to reduce the concentrations of DMF in the daytime. Since the degradation half-life of DMF could be a week or more, continuous daily inputs would lead to buildup of DMF within the cylinder in the absence of any other loss process. However, fugacity-based modelling suggests that advection processes, i.e., rain and wind, are the major factors in determining concentrations in the atmosphere. Even under essentially stagnant conditions, with a wind speed of 1 km/h, the rate of advection of DMF out of the cylinder is so fast that the steady-state concentration would be 0.01 mg/m^3 or less. At a typical average wind speed of 10 km/h, the concentration of DMF in the cylinder would be

reduced by a factor of approximately 100. The EEV of 0.11 mg/m^3 is generally higher than or comparable to measurements made in other countries.

Worst-case estimates of airborne levels in the immediate vicinity of the largest emitter in the sample country (0.11 mg/m^3), which are likely 10- to 100-fold greater than those anticipated under most conditions, do not appreciably exceed the TC (0.1 mg/m^3) derived on the basis of increases in serum hepatic enzymes in exposed workers.

11.1.4 Uncertainties and degree of confidence in human health risk characterization

Quantitative estimates of ambient levels of DMF in the vicinity of point sources in the sample country on which the human health risk characterization is based are highly uncertain (see discussion of uncertainty in section 11.2.3) and likely conservative, although consistent with highest consentrations measured in other countries. The proximity of these predicted concentrations in the vicinity of point sources to residential areas is also unknown. Available monitoring data are inadequate as a basis for characterization of the exposure of the general population to DMF.

There is a high degree of confidence based on studies in both humans and experimental animals that the liver is the target organ for the toxicity of DMF. Cross-sectional studies on hepatic effects in workers, limited principally to males, were complicated by co-exposures to other substances and limitations of available data on exposure, including, in some cases, lack of monitoring data for individuals. However, the levels that induced minimally adverse effects were remarkably consistent across a large number of studies. The TC developed on the basis of increases in serum hepatic enzymes in occupationally exposed populations is likely conservative, since it does not take into account additional exposure by the dermal route.

Although cases of testicular cancer among people exposed to DMF have been reported, these findings have not been corroborated in (limited) epidemiological studies, and it is thus unlikely that DMF is carcinogenic to humans.

11.2 Evaluation of environmental effects

11.2.1 Terrestrial assessment end-points

Since most DMF appears to be released to air in the sample country, and based on the fate of DMF in the ambient environment, biota are expected to be exposed to DMF primarily in air; little exposure to DMF from surface water, soil, or benthic organisms is expected. Based on this, and because of the low toxicity of DMF to

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[1] Notes from N.J. Bunce, University of Guelph, Guelph, Ontario, to A. Chevrier, Environment Canada, dated 1 June 1998.

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a wide range of aquatic and soil organisms, it is unlikely that organisms will be exposed to harmful levels of DMF in Canadian surface waters, soils, or groundwater. Therefore, the focus of the environmental risk characterization will be on terrestrial organisms exposed directly to DMF in ambient air.

Terrestrial plants can be exposed to DMF by direct contact with the atmosphere, but also conceivably by diffusion from raindrops deposited on leaves. No data are available on the toxicity of DMF to terrestrial vascular plants. Seeds, soil fungi, and aquatic angiosperm macrophytes can be used as indicators of the potential sensitivities of trees, shrubs, and other plants. The most sensitive of these organisms appears to be the soil fungus *Sclerotinia homeocarpa*, with an EC_{50} of 4840 mg/litre for growth inhibition (Stratton, 1985). In view of the generally high effect concentrations, it is unlikely that terrestrial plants are particularly sensitive to DMF.

As most DMF is released to air and bioaccumulation is not expected, effects on wildlife will occur mainly through direct exposure by inhalation in the vicinity of the point source. Based on the available information, the home range of common small to medium-sized eastern Canadian mammals is generally much less than 1 km^2 (Banfield, 1974; Burt & Grossenheider, 1976; Forsyth, 1985; US EPA, 1999). By contrast, the home range of the raccoon, a common suburban visitor, is quite variable in size, reportedly ranging from a few square kilometres to thousands of square kilometres (Burt & Grossenheider, 1976; US EPA, 1999). Therefore, small mammals are likely exposed over long periods to highest concentrations of DMF within a few kilometres of the site, while the more mobile medium-sized mammals are probably exposed over time to lower average levels of DMF.

No information has been found on effects of DMF on wildlife. Experimental animals used in laboratory studies will be used as surrogates for small and medium-sized mammals exposed to DMF through inhalation.

11.2.2   Sample environmental risk characterization

The calculation of the EEV is presented in section 11.1.3.

Analysis of exposure pathways and subsequent identification of sensitive receptors are the basis for selection of environmental assessment end-points (e.g., adverse reproductive effects on sensitive fish species in a community). For each end-point, a conservative EEV is selected and an estimated no-effects value (ENEV) is determined by dividing a critical toxicity value (CTV) by an application factor. A hyperconservative or conservative quotient (EEV/ENEV) is calculated for each

of the assessment end-points in order to determine whether there is potential ecological risk.

The long-term (18-month) inhalation LOAEC of 75 mg/m^3 measured for mice is used as a CTV for exposure of small mammals. This value was selected from a large data set composed of acute and long-term studies conducted on a number of laboratory species. Although no direct effects related to survival were observed at the exposure concentrations (up to 1200 mg/m^3), nor were any haematological changes or effects on the estrous cycle observed, the incidence of hepatocellular hypertrophy, hepatic single-cell necrosis, and hepatic Kupffer cell hyperplasia/pigment accumulation was increased at 75 mg/m^3 (Malley et al., 1994). Such effects may not directly manifest themselves as population-level effects in wildlife species; therefore, the ENEV is derived by dividing the CTV by a reduced application factor of 5. This factor also accounts for the extrapolation from a low-effect level to a no-effect level, as well as the uncertainty surrounding the extrapolation from laboratory to field conditions and interspecies and intraspecies variations in sensitivity. As a result, the ENEV is 15 mg/m^3. Therefore, using the EEV of 0.11 mg/m^3, the quotient EEV/ENEV= 0.007. Since this conservative quotient is less than 1, it is unlikely that DMF causes adverse effects on terrestrial organisms in the sample country.

11.2.3   Discussion of uncertainty

There are a number of potential sources of uncertainty in this environmental risk assessment.

The calculated Henry's law constant is uncertain, as solubility cannot be measured. Based on sensitivity analysis, the fugacity-based partitioning estimates can be sensitive to the value used as the Henry's law constant.[1]

Ambient levels near Canadian sources are not available. The EEV was therefore estimated based on available information on releases. This calculated EEV is, however, generally consistent with the highest concentrations measured in other countries. It is unlikely that there are concentrations of DMF in the sample country that are higher than those calculated and used in this assessment. For air, reported releases at the selected location by far exceed reported releases to air at any other location and as such likely constitute a worst-case scenario. For water, concentrations are expected to

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[1] Collection of notes and modelling results submitted by A. Bobra, AMBEC Environmental Consultant, to Chemicals Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, 1999.

be low because of the limited releases identified to this medium and the limited partitioning of DMF from air into water. Small spills and leakage could increase levels of DMF in soil and groundwater; however, the available information suggests that such releases would be small and infrequent.

Regarding effects of DMF on terrestrial organisms, although no toxicity data were identified for vascular plants, data for effects on seeds and aquatic macrophytes suggest that terrestrial vegetation is not particularly sensitive to DMF. Additional evidence of effects on terrestrial plants would strengthen the conclusion that DMF is not expected to damage gymnosperms, angiosperms, and other vascular plants.

There is uncertainty concerning the extrapolation from available toxicity data for laboratory mammals to potential effects on wildlife populations. To account for these uncertainties, an application factor was used in the environmental risk analysis to derive ENEVs.

12. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

IARC (1999) has classified DMF in Group 3 (not classifiable as to its carcinogenicity to humans). There was inadequate evidence for carcinogenicity of DMF in humans. There was evidence suggesting lack of carcino-genicity of DMF in experimental animals.

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N,N-Dimethylformamide

APPENDIX 1 — SOURCE DOCUMENT

Government of Canada (in press)

Copies of the *Canadian Environmental Protection Act Priority Substances List Assessment Report* (Government of Canada, in press) and unpublished supporting documentation for *N,N*-dimethylformamide may be obtained from:

Commercial Chemicals Evaluation Branch
Environment Canada
14th floor, Place Vincent Massey
351 St. Joseph Blvd.
Hull, Quebec
Canada K1A 0H3

or

Environmental Health Centre
Health Canada
Address Locator: 0801A
Tunney's Pasture
Ottawa, Ontario
Canada K1A 0L2

Initial drafts of the supporting documentation and Assessment Report for DMF were prepared by staff of Health Canada and Environment Canada.

The environmental sections were reviewed externally by:

D. Andrews, Golder Associates Ltd.
K. Bolton, University of Toronto
N. Bunce, University of Guelph
R. Gensemer, Boston University
D. Hastie, York University
S. Mabury, University of Toronto
M. Mumtaz, Chinook Group Ltd.
C. Nalewajko, University of Toronto
M. Sheppard, EcoMatters Inc.

Sections of the supporting documentation pertaining to human health were reviewed externally by G. Kennedy, DuPont Haskell Laboratory for Toxicology and Industrial Medicine, to address adequacy of coverage.

Accuracy of reporting, adequacy of coverage, and defensibility of conclusions with respect to hazard identification and dose–response analyses were considered at a panel of the following members, convened by Toxicology Excellence in Risk Assessment on 14 February 2000 in Ottawa, Canada:

M.S. Abdel-Rahman, University of Medicine & Dentistry of New Jersey
C. Abernathy, US Environmental Protection Agency
J.P. Christopher, California Environmental Protection Agency
J.C. Collins, Solutia, Inc.
J.T. Colman, Syracuse Research Corporation
M. Mumtaz, Agency for Toxic Substances and Disease Registry
K.A. Poirier, Toxicology Excellence in Risk Assessment
J.E. Whalen, US Environmental Protection Agency

APPENDIX 2 — CICAD PEER REVIEW

The draft CICAD on *N,N*-dimethylformamide was sent for review to institutions and organizations identified by IPCS after contact with IPCS National Contact Points and Participating Institutions, as well as to identified experts. Comments were received from:

A. Aitio, International Programme on Chemical Safety, World Health Organization, Switzerland

M. Baril, Institut de Recherche en Santé et en Sécurité du Travail du Québec (IRSST), Canada

R. Benson, Drinking Water Program, US Environmental Protection Agency, USA

R.S. Chhabra, National Institute for Environmental and Health Sciences/National Institutes of Health (NIEHS/NIH), USA

R. Hertel, Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Germany

C. Hiremath, US Environmental Protection Agency, USA

H. Kafferlein, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander University Erlangen-Nuremberg, Germany

F. Larese, Institute of Occupational Medicine, University of Trieste, Italy

H. Lendle, Product Safety, BASF AG, Germany

I. Mangelsdorf, Fraunhofer Institute for Toxicolgy and Aerosol Research, Germany

J. Mraz, Centre of Industrial Hygiene and Occupational Diseases, National Institute of Public Health, Czech Republic

P. Ridgeway, Health and Safety Executive, United Kingdom

P. Schulte, National Institute for Occupational Safety and Health, USA

E. Soderlund, Department of Environmental Medicine, National Institute of Public Health, Norway

D. Willcocks, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Australia

P. Yao, Chinese Academy of Preventive Medicine, People's Republic of China

K. Ziegler-Skylakakis, Beratergremium für Umweltrelevante Altstoffe (BUA), Germany

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APPENDIX 3 — CICAD FINAL REVIEW BOARD

Helsinki, Finland, 26–29 June 2000

Members

Mr H. Ahlers, Education and Information Division, National Institute for Occupational Safety and Health, Cincinnati, OH, USA

Dr T. Berzins, National Chemicals Inspectorate (KEMI), Solna, Sweden

Dr R.M. Bruce, Office of Research and Development, National Center for Environmental Assessment, US Environmental Protection Agency, Cincinnati, OH, USA

Mr R. Cary, Health and Safety Executive, Liverpool, United Kingdom *(Rapporteur)*

Dr R.S. Chhabra, General Toxicology Group, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Dr H. Choudhury, National Center for Environmental Assessment, US Environmental Protection Agency, Cincinnati, OH, USA

Dr S. Dobson, Centre for Ecology and Hydrology, Monks Wood, Abbots Ripton, United Kingdom *(Chairman)*

Dr H. Gibb, National Center for Environmental Assessment, US Environmental Protection Agency, Washington, DC, USA

Dr R.F. Hertel, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany

Ms K. Hughes, Priority Substances Section, Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada

Dr G. Koennecker, Chemical Risk Assessment, Fraunhofer Institute for Toxicology and Aerosol Research, Hanover, Germany

Ms M. Meek, Existing Substances Division, Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada

Dr A. Nishikawa, Division of Pathology, Biological Safety Research Centre, National Institute of Health Sciences, Tokyo, Japan

Dr V. Riihimäki, Finnish Institute of Occupational Health, Helsinki, Finland

Dr J. Risher, Agency for Toxic Substances and Disease Registry, Division of Toxicology, US Department of Health and Human Services, Atlanta, GA, USA

Professor K. Savolainen, Finnish Institute of Occupational Health, Helsinki, Finland *(Vice-Chairman)*

Dr J. Sekizawa, Division of Chem-Bio Informatics, National Institute of Health Sciences, Tokyo, Japan

Dr S. Soliman, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

Ms D. Willcocks, National Industrial Chemicals Notification and Assessment Scheme, Sydney, NSW, Australia

Observer

Dr R.J. Lewis (representative of European Centre for Ecotoxicology and Toxicology of Chemicals), Epidemiology and Health Surveillance, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA

Secretariat

Dr A. Aitio, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland *(Secretary)*

Dr P.G. Jenkins, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Dr M. Younes, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

N,N-Dimethylformamide

APPENDIX 4 — BENCHMARK DOSE CALCULATIONS

In subchronic inhalation assays in F344 rats, there was an increase in relative liver weight in females and increased cholesterol in both sexes at 50 ppm (150 mg/m³), with no clear dose–response (LOEC) (NTP, 1992a), progressive histopathological hepatic changes in both sexes at 400 and 800 ppm (1200 and 2400 mg/m³) (Craig et al., 1984), and hepatocellular necrosis in both sexes at 400 ppm (1200 mg/m³) (NTP, 1992a). B6C3F1 mice had hepatocellular hypertrophy at 50 ppm (150 mg/m³) (LOEC), in addition to significantly increased relative liver weight in both sexes without clear dose–response (NTP, 1992a) and hepatic cytomegaly at 150 ppm (450 mg/m³) and higher (Craig et al., 1984). No signs of toxicity were observed in monkeys exposed to up to 500 ppm (1500 mg/m³) (Hurtt et al., 1992).

In a chronic inhalation bioassay in Crl:CD BR rats, at 100 ppm (300 mg/m³), there were significant increases in centrilobular hepatocellular hypertrophy (both sexes), hepatic accumulation of lipofuscin/haemosiderin (both sexes), and hepatic single-cell necrosis (females only). In mice [Crl:CD 1 (ICR)BR], at 25 ppm (75 mg/m³), there was centrilobular hepatocellular hypertrophy (males), hepatic single-cell necrosis (males and females), and hepatic Kupffer cell hyperplasia/pigment accumulation (males) (Malley et al., 1994).

Data on dose–response following ingestion are limited to medium-term exposure studies. At 250 mg/kg body weight per day, liver cell enlargement was reported in Crl:CD rats; at 50 mg/kg body weight per day, relative liver weight was significantly increased in males (Kennedy & Sherman, 1986). In Wistar rats, relative liver weight was significantly increased at 69 mg/kg body weight per day, but no histopathological lesions were observed at doses up to 235 mg/kg body weight per day (Becci et al., 1983). In CD-1 mice, only mild histopathological changes were observed in the liver at 246 mg/kg body weight per day; at 96 mg/kg body weight per day, relative liver weight was significantly increased in females. No adverse effects were observed in beagle dogs administered up to 34.8 mg/kg body weight day in the diet for 13 weeks.

It should be noted that the lowest concentration (50 ppm [150 mg/m³]) at which effects were observed in the liver of rats (NTP, 1992a) in an inhalation assay is equivalent to an intake of 46.5 mg/kg body weight per day in rats,[1] which is consistent with the effects levels in Crl:CD rats (Kennedy & Sherman, 1986) and Wistar rats (Becci et al., 1983) following dietary exposure. The lowest concentration (50 ppm [150 mg/m³]) to which mice were exposed in the NTP (1992a) is equivalent to an intake of 200 mg/kg body weight per day,[2] which is consistent with the effect levels in the dietary assay in mice reported by Becci et al. (1983).

Reported incidence, benchmark concentrations (BMCs) at the 5% level, and associated *P*-values and goodness of fit statistics for effects on the liver for relevant end-points in the most robust medium- and long-term exposure studies for ingestion and inhalation, respectively, are presented in Tables 2 and 3.

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[1] 1 mg/m³ = 0.31 mg/kg body weight per day in rats (Health Canada, 1994)**.**

[2] 1 mg/m³ = 1.33 mg/kg body weight per day in mice (Health Canada, 1994)**.**

For the discrete end-points, the BMC₀₅ is defined as the concentration of chemical that causes a 5% increase in incidence over the background response rate. It is calculated by first fitting the following model to the dose–response data (Howe, 1995):

$$P(d) = q_0 + (1 - q_0) @ [1 - e^{-q_1 d - \dots - q_k d^k}]$$

where d is dose, k is the number of dose groups in the study, P(d) is the probability of the animal developing the effect at dose d, and $q_i > 0$, $i = 1,..., k$ is a parameter to be estimated.

The models were fit to the incidence data using THRESH (Howe, 1995), and the BMC₀₅s were calculated as the concentration C that satisfies

$$\frac{P(C) - P(0)}{1 - P(0)} = 0.05$$

A chi-square lack of fit test was performed for each of the model fits. The degrees of freedom for this test are equal to k minus the number of q_i's whose estimates are non-zero. A *P*-value less than 0.05 indicates a significant lack of fit.

For the continuous end-points, the BMC₀₅ is defined as the dose that causes a 5% increase in the absolute risk of seeing an "adverse" response. This method utilizes the "hybrid" method of Crump (1995), in which the adverse response level in the control group is specified as 5%. That is, 5% of the animals in the control group would, by natural variation, have a response that would be considered adverse. Then, the probability of being adverse, as opposed to the response itself, is modelled.

The Weibull model was fit to each of the end-points using BENCH_C (Crump & Van Landingham, 1996):

$$P(d) = p_0 + (1 - p_0) [1 - e^{(-\$d)^k}]$$

where d is dose, P(d) is the probability of an adverse response at dose d, and k, $\$$, and p_0 are parameters to be estimated. The BMC₀₅ was then calculated as the concentration C such that

$$P(C) - P(0) = 0.05$$

An F-test was used to assess lack of fit of the model. A *P*-value less than 0.05 indicates lack of fit.

N,N-DIMETHYLFORMAMIDE

0457

October 2000

CAS No: 68-12-2 RTECS No: LQ2100000 UN No: 2265 EC No: 616-001-00-X	Dimethylformamide DMF DMFA N-formyldimethylamine C_3H_7NO / $HCON(CH_3)_2$ Molecular mass: 73.09

TYPES OF HAZARD/ EXPOSURE	ACUTE HAZARDS/SYMPTOMS	PREVENTION	FIRST AID/FIRE FIGHTING
FIRE	Flammable. Gives off irritating or toxic fumes (or gases) in a fire.	NO open flames, NO sparks, and NO smoking. NO contact with oxidizing agents.	Powder, alcohol-resistant foam, water spray, carbon dioxide.
EXPLOSION	Above 58°C explosive vapour/air mixtures may be formed.	Above 58°C use a closed system, ventilation.	In case of fire: keep drums, etc., cool by spraying with water.

EXPOSURE		PREVENT GENERATION OF MISTS! AVOID EXPOSURE OF (PREGNANT) WOMEN!	
Inhalation	Abdominal pain. Diarrhoea. Nausea. Vomiting. Facial flushing.	Ventilation, local exhaust, or breathing protection.	Fresh air, rest. Refer for medical attention.
Skin	MAY BE ABSORBED!	Protective gloves. Protective clothing.	Remove contaminated clothes. Rinse and then wash skin with water and soap. Refer for medical attention.
Eyes	Redness. Pain.	Safety goggles, or eye protection in combination with breathing protection.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
Ingestion		Do not eat, drink, or smoke during work.	Rinse mouth.

SPILLAGE DISPOSAL	PACKAGING & LABELLING
Ventilation. Remove all ignition sources. Collect leaking and spilled liquid in sealable containers as far as possible. Absorb remaining liquid in sand or inert absorbent and remove to safe place. (Extra personal protection: complete protective clothing including self-contained breathing apparatus).	T Symbol R: 61-20/21-36 S: 53-45 Note: E UN Hazard Class: 3 UN Pack Group: III

EMERGENCY RESPONSE	STORAGE
Transport Emergency Card: TEC (R)-30G35 NFPA Code: H1; F2; R0	Separated from strong oxidants, halogens.

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0457		**N,N-DIMETHYLFORMAMIDE**

IMPORTANT DATA

Physical State; Appearance
COLOURLESS TO YELLOW LIQUID, WITH CHARACTERISTIC ODOUR.

Chemical dangers
The substance decomposes on heating or on burning producing toxic fumes including nitrogen oxides.
Reacts violently with oxidants, nitrates and halogenated hydrocarbons. Attacks some plastic and rubber.

Occupational exposure limits
TLV: 10 ppm; (skin) (ACGIH 2000).
MAK: 10 ppm; 30 mg/m^3; skin, Re2 (1999)

Routes of exposure
The substance can be absorbed into the body by inhalation and through the skin.

Inhalation risk
A harmful contamination of the air will be reached rather slowly on evaporation of this substance at 20°C.

Effects of short-term exposure
The substance is irritating to the eyes.
The substance may cause effects on the liver, resulting in jaundice. See Notes.

Effects of long-term or repeated exposure
The substance may have effects on the liver, resulting in impaired functions.
Animal tests show that this substance possibly causes toxic effects upon human reproduction.

PHYSICAL PROPERTIES

Boiling point: 153°C
Melting point: -61°C
Relative density (water = 1): 0.95
Solubility in water: miscible
Vapour pressure, Pa at 25°C: about 492
Relative vapour density (air = 1): 2.5

Relative density of the vapour/air-mixture at 20°C (air = 1): 1.00
Flash point: 58°C c.c.
Auto-ignition temperature: 445°C
Explosive limits, vol% in air: 2.2-15.2 at 100°C
Octanol/water partition coefficient as log Pow: -0.87

ENVIRONMENTAL DATA

NOTES

Use of alcoholic beverages enhances the harmful effect.
Resulting symptoms could be delayed from several hours up to several days.
Environmental effects from the substance have been investigated, but none has been found.

ADDITIONAL INFORMATION

LEGAL NOTICE	Neither the EC nor the IPCS nor any person acting on behalf of the EC or the IPCS is responsible for the use which might be made of this information

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RÉSUMÉ D'ORIENTATION

Ce CICAD sur le *N*,*N*-diméthylformamide (DMF) a été préparé conjointement par la Direction de l'hygiène du milieu de Santé Canada et la Direction de l'évaluation des produits chimiques commerciaux d'Environnement Canada, sur la base d'une documentation préparée simultanément dans le cadre du Programme d'évaluation des substances prioritaires, en application de la Loi canadienne sur la protection de l'environnement (LCPE). Les évaluations sanitaires des substances prioritaires effectuées en application de cette loi portent sur les effets que pourraient avoir ces produits sur la santé humaine en cas d'exposition indirecte dans l'environne-ment. L'exposition professionnelle n'est pas abordée dans le document de base. La présente mise au point prend en compte les données sur les effets environne-mentaux jusqu'à septembre 1999 et les données sur les effets sanitaires jusqu'à février 2000. L'appendice 1 donne des informations sur la nature de l'examen par des pairs et sur les sources documentaires. D'autres études ont également été utilisées, à savoir celle l'IARC/CIRC (1999) et celle du BUA (1994). Des renseignements sur l'examen par des pairs du présent CICAD sont donnés à l'appendice 2. Ce CICAD a été adopté en tant qu'évalu-ation internationale lors de la réunion du Comité d'évaluation finale qui s'est tenue à Helsinki du 26 au 29 juin 2000. La liste des participants à cette réunion figure à l'appendice 3. La fiche internationale sur la sécurité chimique (ICSC 0457) du *N*,*N*-diméthyl-formamide, établie par le Programme international sur la sécurité chimique (IPCS, 1999), est également reproduite dans le présent document.

Le *N*,*N*-diméthylformamide (No CAS 68-12-2) est un solvant organique produit en grande quantité dans l'ensemble du monde. On utilise dans l'industrie chimique comme solvant, comme intermédiaire ou comme additif. Il se présente sous la forme d'un liquide incolore dégageant une faible odeur qui rappelle celle des amines. Il est miscible en toutes proportions à l'eau et à la plupart des solvants organiques. Sa tension de vapeur est relativement faible.

Une fois libéré dans l'air, le DMF y demeure en majeure partie jusqu'à décomposition par réaction avec des radicaux hydroxyles. La libération indirecte de DMF dans l'air, notamment à partir d'autres milieux, ne contribue guère au maintien de la concentration de ce composé dans le compartiment atmosphérique. On estime que le DMF présent dans l'air est photo-oxydé en l'espace de quelques jours. Une partie du DMF atmosphérique peut cependant atteindre le milieu aquatique ou terrestre, vraisemblablement à la faveur des précipitations. Le DMF qui passe dans l'eau subit une décomposition *in situ* sans transfert vers d'autres compartiments. Libéré dans le sol, il y demeure en

majeure partie - probablement dans l'eau des pores - jusqu'à dégradation par voie chimique ou biologique. En cas de décharge dans les eaux ou au sol, on peut s'attendre à une biodégradation relativement rapide (demi-vie de 18 à 36 h). Si le composé parvient jusqu'aux nappes souterraines, sa décomposition anaérobie sera lente. Compte tenu du mode d'utilisation du DMF, l'exposition de la population générale à ce composé est vraisemblablement très faible.

Etant donné que dans le pays témoin, la majeure partie du DMF est effectivement libérée dans l'air et compte tenu du devenir de ce composé dans l'environ-nement, l'exposition des organismes vivants est essen-tiellement atmophérique et les organismes benthiques, comme ceux qui peuplent les eaux de surface ou le sol, sont sans doute peu exposés. Compte tenu de cela et étant donné la faible toxicité du DMF pour nombre d'organismes aquatiques ou terricoles, la caractérisation du risque vise essentiellement les organismes terrestres directement exposés au DMF présent dans l'air ambiant.

Le DMF est rapidement absorbé en cas d'expo-sition par voie orale, percutanée ou respiratoire. Une fois absorbé, le composé se répartit de façon uniforme dans l'organisme et après avoir été métabolisé principalement au niveau du foie, il est assez rapidement excrété par la voie urinaire sous la forme de métabolites. La principale voie métabolique consiste en une hydroxylation du groupement méthyle conduisant au *N*-(hydroxyméthyl)-*N*-méthylformamide (HMMF), qui est le principal métabolite urinaire chez l'Homme et l'animal. Le HMMF peut à son tour subir une décomposition en *N*-méthylformamide (NMF), dont l'hydroxylation enzymatique au niveau du groupement *N*-méthyle va entraîner la formation de *N*-(hydroxyméthyl)formamide (HMF), qui se décompose ensuite en formamide. Il existe également une possibilité de bifurcation métabolique à partir du NMF qui consiste en une oxydation du groupement formyle conduisant à la *N*-acétyl-*S*-(*N*-méthylcarbamoyl)cystéine (AMCC), métabolique dont on a décelé la présence dans l'urine humaine et l'urine de rongeurs. Au cours de ce processus, il se forme également un intermédiaire réactif dont la structure n'est pas encore élucidée (peut-être de l'isocyanate de méthyle). Bien qu'on ne dispose pas de preuve expérimentale directe, il se pourrait que ce composé soit le métabolite présumé toxique. A la lumière des données existantes, il semblerait que chez l'Homme, la proportion de DMF métabolisée par la voie présumée toxique soit plus importante que chez l'animal de laboratoire. Il existe une interaction métabolique entre le DMF et l'alcool, qui, bien qu'encore mal élucidée, pourrait être due à l'action inhibitrice de ce composé sur l'alcool-déshydrogénase.

Les données tirées d'analyses de cas individuels ou d'études transversales sur des populations professionnellement exposées, montrent, en accord avec

les résultats de l'expérimentation animale, que chez l'Homme, c'est le foie qui est l'organe-cible du DMF. L'ensemble des effets correspond à ce qui s'observe chez l'animal de laboratoire, c'est-à-dire des troubles digestifs, une intolérance à l'alcool, l'augmentation du taux sérique des enzymes hépatiques (aspartate-amino-transférase, alanine-aminotransférase, γ-glutamyl-transpeptidase et phosphatase alcaline) accompagnés d'anomalies histopathologiques et de modifications ultrastructurales (nécrose hépatocellulaire, hypertrophie des cellules de Kupffer, stéatose microvésiculaire, lysosomes complexes, mitochondries pléomorphes et dégénérescence graisseuse avec présence occasionnelle de lipogranulomes).

A la lumière des données disponibles, il n'existe pas de faits probants ni cohérents qui témoignent d'une augmentation des tumeurs de toutes localisations imputable à l'exposition au DMF sur le lieu de travail. Les cas de cancer du testicule qui avaient été rapportés n'ont pas été confirmés par une étude de cohorte cas-témoins. Pour ce qui est d'autres localisations, aucune augmentation systématique de la fréquence tumorale n'a pu être associée à une exposition au DMF.

En ce qui concerne la génotoxicité du composé pour des populations professionnellement exposées, les données ne sont pas non plus très probantes ni cohérentes, les résultats des études effectuées sur des travailleurs exposés (au DMF et à d'autres composés) étant mitigés. L'ensemble des observations ne cadre pas avec les variations de l'exposition d'une étude à l'autre. Cependant, en raison de la relation dose-réponse positive observée lors de l'étude où cette possibilité avait été explorée, il s'agit là d'un domaine qui mériterait des études supplémentaires, même si les résultats obtenus dans des systèmes d'épreuve expérimentaux sont très largement négatifs en ce qui concerne la génotoxicité du DMF.

La toxicité aiguë du DMF est faible et il n'est que légèrement à modérément irritant pour les yeux et la peau. On n'a pas trouvé de données sur son pouvoir sensibilisateur. Les études de toxicité aiguë ou chronique par administration de doses répétées mettent invariablement en évidence l'hépatotoxicité du DMF, même aux concentrations ou aux doses les plus faibles. Au nombre des effets constatés figurent des modifications touchant les enzymes hépatiques qui sont caractéristiques d'une action toxique, l'augmentation du poids du foie, une dégénérescence histopathologique progressive pouvant conduire à la mort cellulaire et l'accroissement du taux sérique des enzymes hépatiques. Après avoir exposé des rats et des souris par la voie respiratoire et la voie orale, on a constaté l'existence d'une relation dose-réponse pour l'ensemble de ces effets. Par ailleurs, l'ordre de sensibilité des diverses

espèces relativement à ces effets est le suivant : souris > rat > singe.

La base de données relative à la cancérogénicité du DMF ne comporte en tout et pour tout que deux épreuves biologiques sur le rat et la souris, mais il en ressort néanmoins que l'inhalation prolongée de ce composé n'entraîne pas d'augmentation de l'incidence tumorale. Comme on l'a vu, les résultats des tests de génotoxicité sont très largement négatifs; ils proviennent d'études approfondies *in vitro*, consistant notamment à rechercher la présence de gènes mutés, ainsi que d'une base de données plus limitée constituée à partir d'épreuves *in vivo*.

L'expérimentation animale montre que le DMF n'a d'effets nocifs sur la reproduction qu'à des concentra-tions plus fortes que celles qui sont hépatotoxiques, après exposition tant par la voie respiratoire que par la voie orale. De même, lors d'études sur le développement bien conduites et publiées tout récemment, on n'a observé d'effets foetotoxiques et tératogènes systém-atiques qu'aux doses ou aux concentrations toxiques pour la mère.

Les données existantes sont insuffisantes pour permettre une évaluation des effets neurologiques et immunologiques du DMF.

Le présent CICAD et la caractérisation du risque que constitue le DMF ont essentiellement pour objet les effets de ce composé lors d'une exposition indirecte dans l'environnement.

C'est l'air au voisinage de sources ponctuelles de DMF qui fait courir à la population générale le risque d'exposition le plus important. D'après les études épidé-miologiques effectuées sur des travailleurs exposés et les informations tirées de la base de données relativement fournie qui a été constituée à partir des résultats de l'expérimentation animale, c'est le foie qui constitue l'organe cible de l'action toxique du DMF. En se basant sur l'augmentation du taux sérique des enzymes hépa-tiques, on a fixé à 0,03 ppm (0,1 mg/m^3) la concentration tolérable.

On n'a pas trouvé de données sur la toxicité du DMF pour les plantes vasculaires terrestres. Pour les indicateurs de sensibilité potentielle des arbres et des arbustes, les concentrations agissantes sont élevées, aussi est-il peu probable que les végétaux terrestres soient particulièrement sensibles à ce composé. En ce qui concerne les autres organismes terrestres, on est parvenu à une valeur de 15 mg/m^3 pour la concentration sans effet en prenant la valeur limite pour l'hépatotoxicité chez la souris divisée par un coefficient d'application. En comparant cette valeur avec une estimation prudente de l'exposition on peut conclure que

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dans le pays témoin, le DMF n'a vraisemblablement aucun effet nocif sur les organismes terrestres.

RESUMEN DE ORIENTACIÓN

Este CICAD sobre la *N,N*-dimetilformamida (DMF), preparado conjuntamente por la Dirección de Higiene del Medio del Ministerio de Salud del Canadá y la División de Evaluación de Productos Químicos Comerciales del Ministerio de Medio Ambiente del Canadá, se basó en la documentación preparada al mismo tiempo como parte del Programa de Sustancias Prioritarias en el marco de la *Ley Canadiense de Protección del Medio Ambiente* (CEPA). Las evaluaciones de sustancias prioritarias previstas en la CEPA tienen por objeto valorar los efectos potenciales para la salud humana de la exposición indirecta en el medio ambiente general, así como los efectos ecológicos. En este documento original no se abordó la exposición ocupacional. En este examen se analizaron los datos identificados hasta el final de septiembre de 1999 (efectos ecológicos) y febrero de 2000 (efectos en la salud humana). La información relativa al carácter del examen colegiado y la disponibilidad del documento original figura en el apéndice 1. También se consultaron otros exámenes, entre ellos el del IARC (1999) y el del BUA (1994). La información sobre el examen colegiado de este CICAD aparece en el apéndice 2. Este CICAD se aprobó como evaluación internacional en una reunión de la Junta de Evaluación Final celebrada en Helsinki (Finlandia) del 26 al 29 de junio de 2000. La lista de participantes en esta reunión figura en el apéndice 3. La Ficha internacional de seguridad química (ICSC 0457) para la *N,N*-dimetilformamida, preparada por el Programa Internacional de Seguridad de las Sustancias Químicas (IPCS, 1999), también se reproduce en este documento.

La *N,N*-dimetilformamida (CAS Nº 68-12-2) es un disolvente orgánico que se produce en grandes cantidades en todo el mundo. Se utiliza en la industria química como disolvente, intermediario y aditivo. Es un líquido incoloro con un ligero olor a amina. Es completamente miscible con el agua y la mayoría de los disolventes orgánicos y su presión de vapor es relativamente baja.

Cuando se libera en el aire, la mayor parte de las emisiones de *N,N*-dimetilformamida se mantienen en este compartimento, donde se degrada por reacción química con radicales hidroxilo. Las emisiones indirectas de *N,N*-dimetilformamida al aire, por ejemplo por desplazamiento desde otros compartimentos del medio ambiente, desempeñan sólo una pequeña función en el mantenimiento de los niveles de *N,N*-dimetilformamida en la atmósfera. Se estima que la fotooxidación de la *N,N*-dimetilformamida en el aire dura unos días. Sin embargo, parte de la *N,N*-dimetilformamida atmosférica puede alcanzar los medios acuático y terrestre, posiblemente con la lluvia. Cuando se libera *N,N*-dimetilformamida en el agua, se degrada allí y no pasa a otros compartimentos. Cuando se libera al suelo, la mayor parte de

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la *N,N*-dimetilformamida se mantiene allí - posiblemente en el agua intersticial del suelo - hasta que se degrada por reacción biológica y química. Se supone que las emisiones al agua o al suelo van seguidas de una bio-degradación relativamente rápida (semivida de 18-36 h). Si la *N,N*-dimetilformamida alcanza el agua freática, su degradación anaerobia será lenta. Las pautas de uso de la *N,N*-dimetilformamida hacen suponer que la expo-sición de la población general es probablemente muy baja.

Habida cuenta de que en el país de muestra la mayor parte de la *N,N*-dimetilformamida parece que se libera al aire y teniendo cuenta su destino en el medio ambiente, se supone que la biota está expuesta funda-mentalmente a la *N,N*-dimetilformamida del aire; la exposición a la presente en las aguas superficiales, el suelo o los organismos bentónicos se supone que es escasa. Sobre esta base y debido a su baja toxicidad para una gran variedad de organismos acuáticos y del suelo, la caracterización del riesgo ambiental se concentra en los organismos terrestres expuestos directamente a la *N,N*-dimetilformamida del aire ambiente.

La *N,N*-dimetilformamida se absorbe fácilmente tras la exposición oral, cutánea o por inhalación. Después de la absorción, la *N,N*-dimetilformamida se distribuye de manera uniforme, se metaboliza sobre todo en el hígado y se excreta con relativa rapidez como metabolitos en la orina. En la vía principal interviene la hidroxilación de los grupos metilo, produciendo *N*-(hidroximetil)-*N*-metilformamida, que es el principal intermediario urinario en las personas y en los animales. La *N*-(hidroximetil)-*N*-metilformamida se puede descomponer a su vez para formar *N*-metilformamida. Luego, la oxidación enzimática del *N*-metilo de la *N*-metilformamida puede dar lugar a *N*-(hidroximetil)formamida, que a continuación se degrada a formamida. Una vía alternativa para el metabolismo de la *N*-metilformamida es la oxidación del grupo formilo, produciendo *N*-acetil-*S*-(*N*-metilcarbamoil)-cisteína, que ha sido identificado como un metabolito urinario en los roedores y en las personas. En esta vía se forma un intermediario reactivo, cuya estructura aún no se ha determinado (posiblemente metilisocianato); aunque no se han encontrado pruebas experimentales directas que lo respalden, parece que este intermediario es el metabolito supuestamente tóxico. Los datos disponibles indican que la proporción de *N,N*-dimetilformamida que se puede metabolizar por la vía supuestamente tóxica es mayor en las personas que en los animales de experimen-tación. Se ha detectado una interacción metabólica entre la *N,N*-dimetilformamida y el alcohol, lo cual, aunque no se conoce del todo, se puede deber, al menos en parte, a que inhibe la alcohol deshidrogenasa.

Coincidiendo con los resultados de los estudios en animales de experimentación, los datos disponibles de informes de casos y de estudios de muestras represen-tativas de poblaciones expuestas ocupacionalmente indican que en las personas es el hígado el órgano destinatario de la toxicidad de la *N,N*-dimetilformamida. El perfil de los efectos está en consonancia con el observado en los animales de experimentación, habién-dose detectado trastornos gastrointestinales, intolerancia al alcohol, aumento de las enzimas hepáticas en el suero (aspartato aminotransferasa, alanina aminotransferasa, ⊂-glutamil transpeptidasa y fosfatasa alcalina) y efectos histopatológicos y cambios ultraestructurales (necrosis hepatocelular, agrandamiento de las células de Kupffer, esteatosis microvesicular, lisosomas complejos, mitocondrías pleomórficas y cambios en la grasa con lipogranulomas ocasionales).

Teniendo en cuenta los limitados datos dispon-ibles, no hay pruebas sistemáticas convincentes de un aumento del número de tumores en los lugares asociados con la exposición a la *N,N*-dimetilformamida en el entorno ocupacional. Las notificaciones de casos de cáncer testicular no se han confirmado en un estudio de cohortes y de casos y testigos. No se ha observado un aumento constante de tumores en otros lugares asociados con la exposición a la *N,N*-dimetilformamida.

Hay también pocas pruebas sistemáticas convin-centes de genotoxicidad en las poblaciones expuestas ocupacionalmente a la *N,N*-dimetilformamida, con resultados desiguales en los estudios disponibles sobre trabajadores expuestos (a la *N,N*-dimetilformamida y a otros compuestos). La pauta de las observaciones no es coherente con las variaciones de la exposición en los diversos estudios. Sin embargo, a la vista de la relación dosis-respuesta positiva observada en el único estudio en el cual se investigó, convendría estudiar más este aspecto, aunque los datos disponibles sobre genotoxi-cidad en sistemas experimentales son abrumadoramente negativos.

La *N,N*-dimetilformamida tiene una toxicidad aguda baja y una actividad irritante ocular y cutánea entre ligera y moderada. No se identificaron datos relativos a su potencial de sensibilización. En estudios de toxicidad aguda y de dosis repetidas, la *N,N*-dimetilformamida ha sido siempre hepatotóxica, induciendo efectos en el hígado a las concentraciones o dosis más bajas. El perfil de los efectos incluye alteraciones en las enzimas hepáticas características de la toxicidad, aumento de peso del hígado, cambios histopatológicos de degeneración progresiva y a la larga muerte celular, así como aumento de las enzimas hepáticas en el suero. Tras la exposición por inhalación y por vía oral se ha observado una relación dosis-respuesta para estos efectos en ratas y ratones. Se ha detectado una variación de la sensibilidad entre especies para estos efectos, siendo el orden de sensibilidad ratones > ratas > monos.

Aunque la base datos para la carcinogenicidad se limita a dos biovaloraciones debidamente realizadas en ratas y ratones, no se ha registrado un aumento de la incidencia de tumores tras la exposición por inhalación crónica a la *N,N*-dimetilformamida. El valor probatorio para la genotoxicidad es totalmente negativo, basándose en una investigación amplia mediante valoraciones *in vitro,* en particular para la mutación genética, y en una base de datos más limitada *in vivo.*

En estudios con animales de laboratorio, tras la exposición tanto por inhalación como por vía oral la *N,N*-dimetilformamida indujo efectos reproductivos adversos sólo a concentraciones superiores a las asociadas con los efectos adversos en el hígado. Del mismo modo, en estudios fundamentalmente recientes sobre el desarrollo realizados y notificados de manera adecuada, se han observado sistemáticamente efectos citotóxicos y teratogénicos sólo a concentraciones o dosis con toxicidad materna.

Los estudios disponibles no son suficientes como base para la evaluación de los efectos neurológicos e inmunológicos de la *N,N*-dimetilformamida.

Este CICAD y la caracterización del riesgo en la muestra se concentran fundamentalmente en los efectos de la exposición indirecta en el medio ambiente general.

El aire en la proximidad de fuentes puntuales parece ser el principal origen potencial de exposición de la población general a la *N,N*-dimetilformamida. Sobre la base de los resultados de los estudios epidemiológicos de trabajadores expuestos y de los datos justificativos de una base de datos relativamente amplia de investigaciones en animales de experimentación, el hígado es el principal órgano destinatario de la toxicidad de la *N,N*-dimetilformamida. Se ha obtenido una concentración tolerable de 0,03 ppm (0,1 mg/m^3), teniendo en cuenta el aumento de las enzimas hepáticas en el suero.

No se han identificado datos sobre la toxicidad de la *N,N*-dimetilformamida para las plantas vasculares terrestres. Las concentraciones con efecto para los indicadores de una posible sensibilidad de los árboles, los arbustos y otras plantas son altas; por consiguiente, es poco probable que las plantas terrestres sean particularmente sensibles a la *N,N*-dimetilformamida. Para otros organismos terrestres, se ha estimado un valor sin efectos de 15 mg/m^3, basado en un valor critico de la toxicidad para la toxicidad hepática en ratones dividido por un factor de aplicación. La comparación de este resultado con un valor de exposición estimada prudente indica que es poco probable que la *N,N*-dimetilformamida provoque efectos adversos en los organismos terrestres del país de muestra.

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N-Phenyl-1-naphthylamine (No. 9, 1998)

1,1,2,2-Tetrachloroethane (No. 3, 1998)

1,1,1,2-Tetrafluoroethane (No. 11, 1998)

o-Toluidine (No. 7, 1998)

Tributyltin oxide (No. 14, 1999)

Triglycidyl isocyanurate (No. 8, 1998)

Triphenyltin compounds (No. 13, 1999)

Vanadium pentoxide and other inorganic vanadium compounds (No. 29, 2001)

Exhibit 29

Masonic Cancer Center
UNIVERSITY OF MINNESOTA
Comprehensive Cancer Center designated by the National Cancer Institute

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Supplemental List of Materials Reviewed
January 11, 2023

Reports

1. Report of Steven W. Baertschi, PhD.
2. Report of Fengtian Xue, Ph.D.

Literature

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Exhibit 30

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION

COMMISSION ON ELECTROANALYTICAL CHEMISTRY

DIMETHYLFORMAMIDE: PURIFICATION, TESTS FOR PURITY AND PHYSICAL PROPERTIES

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Analytical Chemistry Division

Commission on Electroanalytical Chemistry

DIMETHYLFORMAMIDE: PURIFICATION, TESTS FOR PURITY AND PHYSICAL PROPERTIES[+]

N,N'-Dimethylformamide (DMF) is a good solvent for organic and to a lesser extent inorganic compounds. It is, together with dimethylsulfoxide and acetonitrile, one of the most widely used of the so-called dipolar aprotic solvents. Owing to its fairly high dielectric constant, it is a moderately dissociating solvent for electrolytes. Acid-base reactions as well as thermodynamic properties of electrolyte solutions have been studied by many authors. Contrary to the N-methylamides it is a typically weakly associated solvent, as seen (Ref. 1) from dielectric studies (the Kirkwood g factor is about one at all temperatures).

Owing to its electron-donor character, DMF reacts with many acids. For example, Gutmann's donicity number (Ref. 2) is 27. Its polarographic range is quite large, e.g., 3.5 V at the dropping mercury electrode with 0.1 M Bu_4NClO_4 as supporting electrolyte (Ref. 3). It is therefore widely used as a solvent for electrochemical reactions, especially reductions.

Pure DMF is colorless and, at room temperature, odorless. It is subject to thermal as well as photochemical degradation. In presence of water, DMF is slowly hydrolyzed according to the equation:

$$HCON(CH_3)_2 + H_2O \longrightarrow HCOOH + (CH_3)_2NH$$
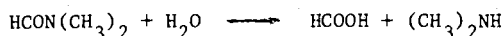
Formic acid and dimethylamine are thus predominant impurities in DMF and determine the odor of the impure solvent. They are weakly acidic and weakly basic respectively; therefore, partial ionization does occur:

$$HCOOH + (CH_3)_2NH \rightleftharpoons HCOO^- + (CH_3)_2NH_2^+$$
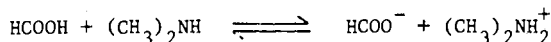
and results in a buffered solution (pH 11) with an increase in the conductivity of the solvent.

Thermal degradation produces dimethylamine and carbon monoxide. Hydrogen (Ref. 4) and hydrogen cyanide (Ref. 5) have been identified among the products of the photochemical degradation of the solvent.

Strongly basic media are difficult to obtain in DMF; there is, to our knowledge, no substance behaving as a strong base in DMF. If autoprotolysis of the medium actually occurs, the anion of the solvent must be very unstable (Ref. 6). It has been claimed (Ref. 7 and 8) that the autoprotolysis constant is smaller than 10^{-25} but no definite value has yet been proposed.

Attention must be paid to the fact that DMF has toxic effects, particularly on the liver and kidneys; the threshold value for air has been fixed (Ref. 9) at 30 mg/m^3.

PURIFICATION OF DIMETHYLFORMAMIDE

Good quality DMF is commercially available. As noted by Vaughn (Ref. 10), spectrograde solvent is not always suitable for all purposes. As a consequence of hydrolysis, the residual water content of commercial DMF is frequently low (0.1%). Many procedures have been proposed and used for the purification of the solvent. Four types of successive operation can be distinguished: treatment with a drying agent, neutralization of basic or acidic impurities, careful distillation, and elimination of gaseous impurities.

---

1. <u>Preparing water-free solvent</u>. Although the boiling point of water is far from that of DMF it is not possible to obtain a dry solvent by distillation only.

One of the first methods proposed for preliminary drying (Ref. 11) was azeotropic distillation with about 10% by volume of dry benzene; the benzene-water azeotrope is removed by distillation at atmospheric pressure. To prevent decomposition the temperature is maintained below 80°C. Alternatively, molecular sieves can be used. The solvent is kept in contact for periods ranging from 1 to 4 days with 4 Å (Ref. 12-15) or 5 Å (Ref. 16) sieves which are removed and replaced from time to time. Ritchie (Ref. 17) recommends the use of Linde AW-500 molecular sieves in 1/16-inch pellets. Studying drying efficiency, he finds that the water content is less than 18 ppm after 27 hours. Molecular sieves can be dried before use by heating in a quartz tube under a stream of argon at 375°C for 24 h (Ref. 18). Finally, a procedure which uses chromatographic purification through alumina has been described by Moe (Ref. 13) in some detail. "A column approximately 100 cm long and 5 cm wide will contain 1 kg of alumina, sufficient for the purification of about 10 l of DMF". After bubbling of pure nitrogen for several hours the DMF thus obtained is thought to be convenient for polarographic use.

In our opinion these three types of operation can be considered only as a first step in drying the solvent, and mild chemical drying agents must also be used. These range from anhydrous BaO (Ref. 11 and 19) to $MgSO_4$ (Ref. 20), Na_2CO_3 (Ref. 6 and 20), or $CuSO_4$ (Ref. 21). Surprisingly good samples of DMF can be obtained using storage of solvent over these chemicals for at least 24 h. It has been recommended that the drying agent be changed at least twice and the container shaken, if not continuously, at least from time to time. It also has been recommended that such an operation is performed in a cold, dark room. As far as Na_2SO_4 or Na_2CO_3 are concerned, the resulting solvents are of about the same quality (Ref. 20). Little or no degradation of the solvent (as estimated through the concentration of dimethylamine) results from such treatment (Ref. 11).

Some of the more common drying agents react with the solvent itself to produce significant amounts of acidic or basic impurities. BaO, cited previously, belongs to this category if it is used at temperatures above 30°C (Ref. 11). Other reagents are potassium hydroxide, calcium hydride (Ref. 5 and 22) and phosphorus pentoxide (Ref. 17, 23 and 24). P_2O_5 is the most frequently used, CaH_2 is probably the most efficient. Prue and Sherrington (Ref. 23) have shaken DMF for three days with P_2O_5, adding each morning about 10 g of fresh reagent. Recently, drying of amides using Vitrid, sodium bis(methoxy-2-ethoxy)aluminohydride, has been recommended (Ref. 25). In DMF it allows attainment of very basic media (pH 30). However, distillation of the solvent from the mixture obtained has not been attempted and is probably very hazardous. Whatever the method used, it is important to proceed with these operations in a dark room or apparatus to prevent any photochemical degradation.

2. <u>Neutralization</u>. Depending on the drying agent used, it has been recommended that the basic or acidic impurities produced are neutralised, either by shaking with picric acid (Ref. 20) or KOH pellets (Ref. 24). This last treatment is particularly recommended after drying over P_2O_5 which generates formic acid. Such neutralization can be done either before or after a first distillation.

3. <u>Distillation</u>. The drying process can be further carried out during this operation. The DMF is refluxed and distilled from P_2O_5 or CaH_2. However, owing to a degradation process increased by heating, it is preferable first to decant the solvent and transfer it under dry nitrogen, and then to distil it at reduced pressure.

The quality of the final product is greatly affected by the care with which the distillation is carried out. It seems to be important to work under vacuum, with a darkened column, or in a pure nitrogen or argon atmosphere. As a rule, the temperature must be kept under 60°C; heating must be gentle and overheating avoided. Distillation in daylight results in the production of hydrogen cyanide (Ref. 5), particularly in the presence of CaH_2. No traces of HCN are detected if the operations are conducted in the dark.

Types of distillation apparatus currently described in the literature do not seem to be very efficient. It is not surprising to note that the best quality DMF, if conductivity is accepted as a test of purity, has been obtained by Brummer (Ref. 12), who used only molecular sieves as drying agents, but carried out the distillation in a slow current of dry nitrogen at low pressure (2 torr) and an efficient column (1 meter packed with Fenske helices). The use of a long column (60 cm at least) with good packing and reflux is recommended. For example, Tanaka (Ref. 21) distilled DMF which had been dried over anhydrous $CuSO_4$ at a pressure of 5 torr through an adiabatic fractional distillation column which was 1.3 cm in diameter, 120 cm in length and packed with helipack coils. Dry nitrogen was passed through the apparatus during the distillation; 60% of the distillate was collected. The conductivity was lower than 1×10^{-7} Ω^{-1} cm^{-1} (25°C). Boiling temperatures at various pressures are given in Table 1.

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TABLE 1. Recommended values for physical constants of DMF at 25°C and 1 atm (except where noted otherwise)

Boiling temperature	T_B	152.3°C (Ref. 47)
		79°C at 61-62 torr (Ref. 37)
		55-56°C at 25-26 torr (Ref. 40)
		34°C at 2-3 torr (Ref. 15)
Melting temperature	T_M	-61°C
Refractive index (Ref. 44)	n_D^{25}	1.42689
Dielectric constant	D	37.0
Surface tension (Ref. 45)	σ	37.1 dyne/cm
Viscosity (Ref. 23)	η	0.00796 poise
Density	ρ	0.9440 g cm^{-3}
Molal volume	V	77.39 cm^3
Heat capacity at constant pressure (Ref. 44)	C_p	37.4 cal/mol
Cubic expansion coefficient	α_p	1.00 x 10^{-3} K^{-1} *
Adiabatic compressibility coefficient (Ref. 44)	β_S	6.1 x 10^{-5} atm^{-1}
Isothermal compressibility coefficient	β_T	6.3 x 10^{-5} atm^{-1} *

*Calculated from data in Ref. (12)

4. Elimination of gaseous impurities. A flow of pure dry nitrogen or argon is passed through the solvent for several hours, in order to eliminate oxygen, carbon monoxide and carbon dioxide. Such a solvent can then be used for polarographic purposes. A more complete deaeration can be achieved using a vacuum line.

5. Conclusions and recommendations. As various authors used different starting materials, it is difficult to compare the efficiency of the various methods of purification. Comparison between different ways of treating the same batch of solvent can be found to our knowledge in only two papers (Ref. 11 and 20). Thomas and Rochow (Ref. 11) always used first azeotropic distillation with benzene and compared subsequent treatment with MgSO$_4$, BaO, alumina and triphenylchlorosilane, followed in each case by distillation. Comparisons were made in terms of specific conductance and water content. Barium oxide as well as alumina treatment meet rather well these two criteria and do not have any side effects, such as producing dimethylamine or HCN. Juillard (Ref. 20) compared drying with Na$_2$CO$_3$, Na$_2$SO$_4$ or molecular sieves with azeotropic distillation with benzene and distillation over P$_2$O$_5$. As far as conductivity and water content are concerned, the different batches of solvent thus obtained were of about the same quality, except that P$_2$O$_5$ has the disadvantage of promoting degradation of the solvent and thus of decreasing the efficiency of the distillation; therefore the use of P$_2$O$_5$ is not recommended. As a confirmation it can be noted that authors using P$_2$O$_5$ or CaH$_2$ as drying agents did not obtain purer solvents than those who employed BaO or Na$_2$CO$_3$ or even only molecular sieves.

It is therefore recommended that use is made first either of azeotropic distillation with benzene, as suggested by Thomas (Ref. 11), or of treatment with molecular sieves, as suggested by Ritchie (Ref. 17), and that the resulting DMF is then shaken with Na$_2$CO$_3$ or, better, with BaO for 1 or 2 days. After decantation the DMF is distilled twice under nitrogen (pressure <15 torr) using a 1-m column. All these operations must be carried out in the dark. After deaeration the solvent is stored under nitrogen and used as soon as possible.

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TESTS FOR PURITY

Owing to its various modes of degradation (hydrolysis, thermal and photochemical decomposition) the principal impurities found in DMF are: dimethylamine, formic acid, hydrogen cyanide, carbon dioxide and carbon monoxide. To this list must be added: water, oxygen, which is quite soluble, and impurities resulting from the purification process.

Conductivity. As stressed earlier, hydrolysis as well as decomposition results in ionic impurities: dimethylammonium formate, carbonate or cyanide. Thus, the conductivity of the solvent is a very good test of its purity.

Experimental conductivities recorded in DMF are always higher than those reported for other aprotic solvents such as ketones or nitriles. According to a rough estimate, the theoretical conductivity of the solvent should be below 10^{-13} $\Omega^{-1}cm^{-1}$. In fact, conductivities obtained by the most careful workers are scarcely ever less than 10^{-7}. The best values have been reported, to our knowledge, by Brummer (Ref. 12) who used for conductometric studies a solvent having conductivities varying from 2 x 10^{-8} to 5 x 10^{-8} Ω^{-1} cm^{-1}. Values below 5 x 10^{-7} have been reported by numerous authors and any batch of DMF which is more conducting can be considered to be impure.

Water. Water can be titrated by the Karl Fischer (K-F) reagent. Kanatharan (Ref. 22) recommends that the titration is conducted slowly, since K-F reagent reacts only slowly with small amounts of water.

Usual procedures do not allow the determination of less than 10 ppm of water. According to Muroi (Ref. 26) it is possible to titrate as little as 0.2 ppm by increasing the sharpness of the end point, using the following procedure: "Add a 10-30 ml sample to 25 ml MeOH containing 8% of a pyridine-SO_2 solution (320 g $SO_2/1$ pyridine) and titrate potentiometrically with K-F reagent having a titre of 0.1-0.5 mg H_2O/ml". The use of DMF as a solvent for K-F reagent also has been advocated (Ref. 27).

Prue (Ref. 23) has titrated water in DMF using triphenylsilyl chloride, from which, according to Thomas (Ref. 11), hydrogen chloride is liberated quantitatively by water (amines or acids are thought to interfere); the HCl content is then estimated from the conductivity of the solution.

It is quite easy to prepare a solvent which contains less than 50 ppm of water. Very low concentrations (< 5ppm) are more difficult to attain. The best value, less than 3 ppm, has been reported by Libbey and Stock (Ref. 28).

Dimethylamine. Colorimetric methods have been used by some authors. In our opinion, as long as the autoprotolysis constant of the solvent is not known, it is not possible to say exactly what is basic and what is acidic in DMF. Kolthoff (Ref. 24) has used p-nitrophenol in the colorimetric determination of total basicity, but specific determinations would be preferable.

Thomas and Rochow (Ref. 11) have based the determination of the amine content on the fact that dimethylamine forms with 1-fluoro-2,4-dinitrobenzene a complex which absorbs in the visible spectrum at 3812 Å. Solvent prepared by Chang and Criss (Ref. 29) was found to contain less than 1 ppm of dimethylamine using this method.

Another spectrophotometric method which allows the determination of the dimethylamine content down to 2 ppm with an error of +10% has been proposed by Pribyl (Ref. 30); dimethyldithiocarbamate, which absorbs at 445 nm, is formed by adding CS_2 and $Cu(AcO)_2$ to an EtOH-pyridine mixture.

Chromatography was thought by Butler (Ref. 18) not to be a reliable means of establishing the organic impurity content of the solvent since DMF can decompose or hydrolyze at high temperatures. Nevertheless, careful studies of the proper experimental conditions have been undertaken (Ref. 31 and 32). In the paper by Filippov (Ref. 32) it is shown that dimethylamine can be determined in DMF at levels as low as 1 ppm using tetrahydroxyethylenediamine as a stationary phase, polysorb-1 as a solid support and a column temperature of 75°C.

Dimethylamine is not electroactive with mercury but can give coordination compounds with cations which will affect the course of electrochemical reductions.

Formic Acid. In contrast to dimethylamine, formic acid is electroactive. Kanatharan and Spritzer (Ref. 22) have attributed to formic acid two peaks, one cathodic, the other anodic, which appear in cyclic voltammograms of aqueous dimethylformamide. Alternating current polarography (Ref. 33), and, better, pulse polarography, can be used to estimate the formic acid content.

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Formic acid can also be determined by titration with a base. Potentiometric titration is
preferred since it allows determination of the dimethylammonium formate content as well.
Megliskii (Ref. 34) has titrated potentiometrically formic acid, dimethylamine and dimethyl-
ammonium formate in DMF using two solutions: 0.1 M $HClO_4$ and 0.1 M KOH, both in alcohol.
Such a method is suitable only for concentrations of the order of at least 100 ppm.

Hydrogen Cyanide. Trisler et al. (Ref. 5) reported the presence of HCN in DMF distilled
over CaH_2 in natural light. Concentrations ranged from 10^{-5} to 10^{-3} M. Spectrophotometric
titration can be carried out with 4-nitrobenzil, which reacts with cyanide ion to form a deep
violet ion.

Oxygen. Oxygen is rather soluble in DMF. A study of oxygen solubility in relation to the
oxygen content of the gas phase has been made by James (Ref. 35). When the gas phase was
air and pure oxygen, the solubility was 2.2×10^{-3} and 3.1×10^{-3} M, respectively.

Oxygen is an electroactive impurity which interferes in polarography and other electrochemi-
cal processes. Two waves are observed (Ref. 36) with $E_{1/2} = -0.8$ and -2.8 V vs. SCE; the
first corresponds to the reduction of oxygen to superoxide:

$$O_2 + e^- \longrightarrow O_2^{\cdot -}$$
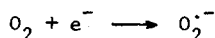
and the second one to the reduction of superoxide to peroxide ion:

$$O_2^{\cdot -} + e^- \longrightarrow O_2^{=}$$
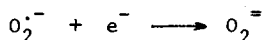
James (Ref. 35) has proposed two methods for the determination of the oxygen concentration;
polarography and the Winkler method. Polarographic measurements are made at -1.2 V vs. SCE,
in order to ensure that the measured diffusion current is not influenced by a polarographic
maximum. A modified Winkler method allows concentrations as low as 10 ppm to be determined.
It depends upon quantitative oxidation of iodide ion to iodine. Such a process is described
in some detail (Ref. 35).

PHYSICAL PROPERTIES OF DIMETHYLFORMAMIDE

Numerical values of physical constants are highly dependent on the purity of the solvent.
Consequently, important discrepancies are found in the literature. The present recommended
values result from a careful examination of three aspects: accuracy of the measurements,
consistency of the data of various authors at different temperatures, and purification of
the solvent. Such a choice is subject to personal evaluation and it seems prudent to give
also the other references.

Density. The density is probably a good criterion of the purity of the solvent. Contamina-
tion with water increases the density (Ref. 23). The following values of the density at
25°C have been found (Ref. 23,8,37,12): 0.9439, 0.9440_2, 0.9441_5 and 0.9442 g cm^{-3}, respec-
tively. Old values greater than 0.9443 frequently found in tables are probably too high.
New work by Kawaizumi and Zana (Ref. 38) seems to indicate that the density of the pure sol-
vent is lower. These authors obtain values ranging from 0.94360 to 0.94368. It is our feel-
ing that these data are more accurate than previous ones but such a low value ($\rho = 0.94364 \pm$
0.00004) must be confirmed by others before being accepted.

Values at various temperatures other than those appearing in Table 2 have been given by
Gopal and Rizvi (Ref. 39). At 20°C Saphon (Ref. 40) has obtained $\rho = 0.94878$ g cm^{-3}, in good
agreement with the value in Table 2.

TABLE 2. Recommended values for physical constants of DMF at various temperatures

		ρ g cm^{-3}	η poise	D
Temperature	20°C	0.9488	0.00845	38
	30°C	0.9394	0.00746	36.1
	40°C	0.9298	0.00664	34.4
	50°C	0.9202	0.00598	32.8
Reference		12	49	1

Viscosity. Other values can be found in References (29) and (41). Prue's data at 25°C are
confirmed by measurements reported by Ames and Sears (Ref. 42).

<u>Dielectric constant</u>. Data given by Bass and Cole (Ref. 1) are preferred to previous results (Ref. 43) of Leader and Gormley (36.71 at 25°C). The value reported at 25°C is interpolated from measurements at various temperatures. Data of Saphon (Ref. 40) are in good agreement with the value reported in Table 2 at 20°C (D = 38.13).

<u>Miscellaneous</u>. Data at various temperatures concerning refractive index, surface tension and isothermal compressibility can be found in Refs. (44), (45) and (12), respectively. Other data concerning thermodynamic properties are reported in Refs. (39) and (44). Plots of vapor pressure, heat of vaporization, heat capacity, density, viscosity, surface tension and thermal conductivity for a large range of temperature have been drawn up by Gallant (Ref. 46). The solubilities of some sixty substances in DMF have been tabulated (Ref. 50). Organic reactions in or with DMF have been summarized (Ref. 51).

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Exhibit 31

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J.C.S. Perkin II

Nitrosative Dealkylation of Some Symmetrical Tertiary Amines

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The rates of nitrosodealkylation of several symmetrical tertiary amines R_3N including trialkylamines (R = Me, Et, Pr[n], and Bu[n]) and substituted trialkylamines (R = $C_6H_5CH_2$, triethanolamine, and nitrilotriacetic acid) have been measured in aqueous acetic acid–acetate buffers. The rate of formation of diethylnitrosamine was found to be first order in nitrous acid, triethylamine, and in the hydrogen ion concentration for pH >3.1. Rates increased with decreasing amine basicity. The rate equation was consistent with rapid, reversible nitrosation by nitrous acid or acetyl nitrite and a rate-determining subsequent elimination.

THE formation of nitrosamines from the reaction of nitrites with tertiary amines was, at one time, discounted. The history of the changing understanding of this reaction system has been reviewed by Hein [1] and by Smith and Loeppky.[2] More recently attention has been directed [3-13] to the public health aspects of the nitrosation of tertiary amines and quaternary ammonium compounds. It has been shown that a wide variety of tertiary amines can react with nitrite in the pH range 3—6.5 and temperature range 37—90° to produce nitrosamines in varying yields. It is claimed [10] that the nitrosation of simple aliphatic tertiary amines in dilute aqueous solution at 100° obeys the kinetic equation (1).

$$\text{Rate} = k[\text{amine}][\text{nitrite}]^3 \qquad (1)$$

This is the only published kinetic study. It seems probable that at these temperatures there will be substantial decomposition of the nitrous acid. A third-power dependence on nitrite concentration is unusual and contrasts with the rate equations obtained for the nitrosation of secondary amines.[14,15] The objectives of the work described here were to define the stoicheiometry and the kinetics of dealkylative nitrosation of some symmetrical tertiary amines in order to judge whether there were some amines which could be subject to this reaction *in vivo*.

EXPERIMENTAL

Materials.—All tertiary amines, with the exception of trimethylamine, were commercially available samples. They were analysed by g.l.c. prior to use. Triethylamine was also checked for removal of any diethylamine by using the procedure of Schweinsberg and Sander.[10] It was noted that there was no difference in the rate of formation of diethylnitrosamine when the purified amine was employed as compared with untreated triethylamine. Trimethylamine was prepared in aqueous solution from reaction of trimethylamine hydrochloride with the stoicheiometric quantity of aqueous sodium hydroxide.

Dialkylnitrosamines were prepared from nitrosation of the secondary amine under standard conditions with the exception of dibenzylnitrosamine where the method of Curtius and Franzen [16] was used. The purity was checked by g.l.c. and t.l.c. prior to spectrophotometric analysis to obtain the molar extinction coefficient.

Qualitative Analysis of the Products of Nitrosation of Triethylamine.—A thoroughly degassed mixture of sodium nitrite and triethylamine dissolved in a sodium acetate–acetic acid buffer solution was heated at 80—90 °C for 2 h and the gaseous products removed by vacuum distillation. Analysis of the products by g.l.c. and i.r. showed the presence of nitrous oxide, acetaldehyde, and diethylnitrosamine.

Determination of the Stoicheiometry of the Nitrosation of Triethylamine.—Nitrosation of triethylamine was carried out at 80 °C for 8 h using reactants as above in a reflux system with an attached trap containing ethanol-free chloroform to absorb volatile products. On termination of the heating, the cooled solution was extracted four times with ethanol-free chloroform including that from the trap and a 10 cm³ portion of the extract was placed in a standard flask with known amounts of the internal g.l.c. standards carbon tetrachloride and hexadecane. G.l.c. analysis was then carried out using an F and M gas chromatograph (6 ft × 1/4 in column of 10% Carbowax 20M on 60—80 mesh Diatoport W with hydrogen as carrier gas). Temperature conditions were injection port 250, detector 310, column 95 (acetaldehyde and carbon tetrachloride), or 180 °C (diethylnitrosamine and hexadecane).

Kinetic Method.—The reaction mixture was contained in a flask fitted with a thermometer, a reflux condenser with an attached chloroform-containing trap, and a Drechsel bottle head for the withdrawal of samples. The flask, containing buffer solution and amine (100 cm³), was immersed in the thermostatted water-bath until temperature equilibration was achieved. Sodium nitrite solution (100 ml) at the same temperature was added with shaking to ensure mixing. Samples for analysis were withdrawn at regular intervals and the reaction was quenched by cooling to 0 °C. When the kinetic run was completed, the samples were allowed to reach room temperature and 10 ml of each were taken, saturated with potassium carbonate, and extracted with a small quantity of chloroform, this sample then being made up to 10 ml with chloroform. The u.v. spectrum of each chloroform solution of the dialkylnitrosamine was measured and the concentration of the nitrosamine determined from the optical density at the absorption maximum of nitrosamine. Prior calibrations were made for each nitrosamine studied. Unless stated otherwise all measurements were carried out with a solution of anhydrous sodium acetate (8.5 g) in 60% aqueous acetic acid (100 ml) as buffer providing an initial pH of the mixture of 3.8—3.9.

RESULTS

Reaction Stoicheiometry.—It was shown for triethylamine that 0.9 ± 0.1 mol of acetaldehyde were formed for each mol of diethylnitrosamine formed in good agreement with

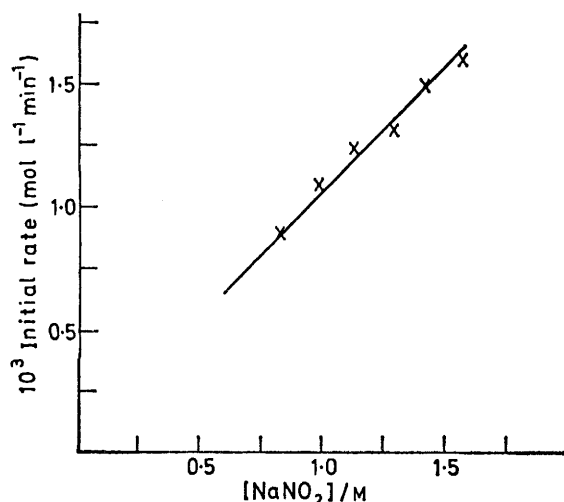**1979**   **1111**

FIGURE 1 Variation of initial rate with nitrite concentration
at 74.9 °C and pH 3.8

the stoicheiometry implied by the results of Smith and
Loeppky.[2]

Kinetic Studies.—The thermal decomposition of nitrous
acid accompanying nitrosodealkylation of the amines pre-
cluded the use of integrated rate equations. The less
accurate initial rate method had to be employed in which
the initial rate is obtained from the slope extrapolated to
zero of plots of the nitrosamine concentration against time.

(1) *Triethylamine.* Figures 1—4 give the data for pro-
duction of diethylnitrosamine. In each set of data one
variable (amine concentration, nitrite concentration, pH,
and temperature) is altered. The variation of the initial
rate of formation of nitrosamine with the stoicheiometric
concentration of amine and nitrite shows that equation (2)
holds.

$$d[Et_2NNO]/dt = k[\text{triethylamine}][NaNO_2] \qquad (2)$$

holds. The actual concentrations of triethylamine and of
the nitrous acid present at a given pH were calculated using
pK_a values of 10.7 for triethylamine and 3.4 for nitrous acid.

$$d[Et_2NNO]/dt = k'[Et_3N][HNO_2] \qquad (3)$$

The rate constants k' obtained in this way are defined by
equation (3) and depend on the pH. The variation of k'
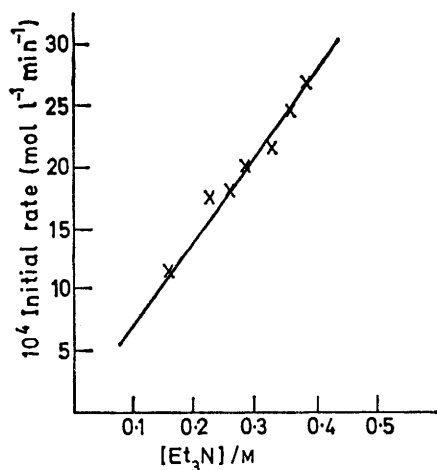
FIGURE 2 Variation of initial rate with triethylamine
concentration at 74.9 °C and pH 3.8

with pH shows that there is a maximum rate at pH 2.9—3.0.
In the pH range 3.1—3.9 this rate coefficient is proportional
to the hydrogen concentration as indicated by Figure 5.
Thus expression (3) can be expanded for this range to give
(4) where k'' is a pH independent rate constant. An

$$d[Et_2NNO]/dt = k''[H^+][Et_3N][HNO_2] \qquad (4)$$

investigation of possible anion catalysis was confined to the
addition of (a) 0.32M-sodium chloride which gave a reaction
mixture of pH 3.70 and no significant increase in the initial
rate of nitrosation and (b) 0.32M-potassium thiocyanate
which gave a reaction mixture of pH 3.95 and a definite
increase in the initial rate of nitrosation accompanied by an
orange colouration.
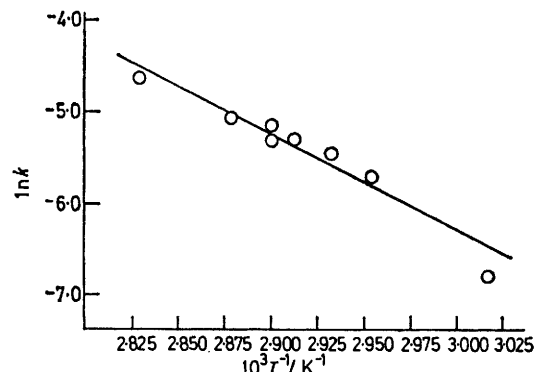
FIGURE 3 Arrhenius plot for the nitrosodealkylation of
triethylamine at pH 3.8
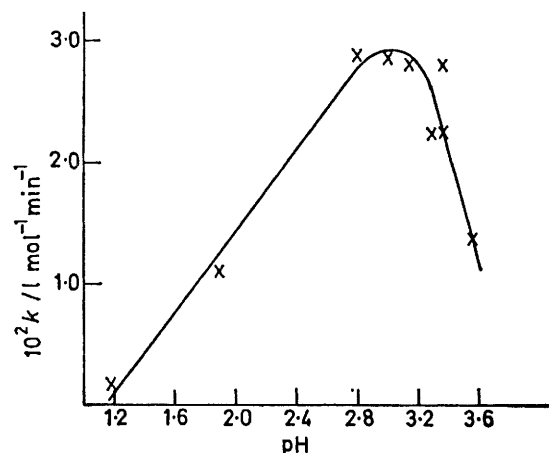
FIGURE 4 Variation of initial rate with pH at 74.8 °C

The activation energy for the nitrosation reaction was
obtained from the Arrhenius plot (see Figure 3) giving a
value of E of 84.8 kJ mol^{-1} for the temperature range
58—81 °C. The temperature dependence of pK_a was neg-
lected.

Other symmetrical tertiary amines R_3N. It was shown for
one further example (R = Bu^n) that the rate of the dealkyl-
ation reaction was first order in both the amine and the
nitrite. The rates for all other amines were therefore
evaluated with the assumption that the empirical rate
equation (2) was obeyed. All these studies were con-
ducted at 75° and pH 3.8, the reaction medium consisting
of 30% acetic acid buffered by sodium acetate (8.5 g). The
concentrations of sodium nitrite required to obtain con-

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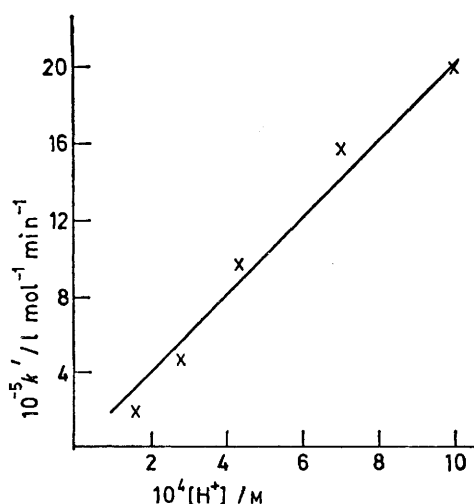
FIGURE 5   Variation of k' with the hydrogen ion
concentration at 74.8 °C

veniently measureable rates were between 0.1 and 0.5M,
amine concentrations varied from *ca.* 3×10^{-2}M for the
most reactive tribenzylamine to 0.15M for the less reactive
strongly basic amines. The results obtained are given in
the Table and compared with the pK_a values of the
amines.[17,18]

Rate constants for the dealkylative nitrosation of tertiary
amines R_3N at 75 °C and pH 3.8,

R	$\varepsilon_{max.}$ [a]/ $l \, mol^{-1} \, cm^{-1}$	pK_a [b]	$10^3 k/$ $l \, mol^{-1} \, min^{-1}$	$10^{-8} k''/$ $l^2 \, mol^{-2} \, min^{-1}$
Et	89.3	10.7	6.4	11
Me	96.4	9.75	8.1	1.6
Pr^n	103	10.7	22	39
HO(CH$_2$)$_2$	83.1	<9.5	25	<2.8
Bu^n	91.1	10.3	30	21
PhCH$_2$	80.4	~8.75	32	~0.63
HO$_2$CCH$_2$	84.0	<9.0	40	<1.4

[a] Extinction coefficients of n,π^*-maxima in chloroform used
for the assay of dialkylnitrosamines. [b] Literature values [17]
and estimates based on basicity series [18] and extrapolations.

DISCUSSION

The earlier work on the nitrosative dealkylation of
tertiary amines was interpreted [1,2,19] in terms of an
initial nitrosation of the amine with formation of an
N-nitrosotrialkylammonium ion and a subsequent *cis*-
elimination of nitroxyl to form a tertiary ammonium ion
$R_2\overset{+}{N}$=CHR'. The end products, a carbonyl compound
and a dialkylnitrosamine, were thought to arise from the
hydrolysis of the ammonium ion and the nitrosation of
the resulting intermediate secondary amine.[2] The
stoicheiometry observed for triethylamine in this work
is consistent with such a mechanism. The rate is
proportional to the hydrogen ion and stoicheiometric
nitrite and triethylamine concentrations in the pH >3
range. The concentration of nitrous acid and of the
unprotonated amine are related to these stoicheiometric
concentrations by pH-dependent dissociation equilibria,
but the rate constants based on the true HNO$_2$ and
amine concentrations are still proportional to the
hydrogen ion concentration (Figure 5). Equation (3)

can therefore be rewritten for the specified acidity range
in the more generalised form (4) using the pH inde-
pendent rate constant k''.

Equation (4) resembles previously established rate
laws for the kinetics of nitrosation or diazotisation of
amines.[20] These usually are the result either of slow
production of the nitrosating agent or rate-determining
nitrosation steps. Both high temperatures and high
concentrations of nitrite are required to obtain adequate
rates for the nitrosodealkylation reaction. In some of
the runs the total initial concentration for the sum of
the various nitrosating species exceeds 0.2M. We can
therefore rule out the possibility that the rate of this
reaction is limited by slow production of the nitrosating
agent, within the pH range investigated, and a rate-
determining nitrosation may seem more likely. Such a
situation is commonly encountered in the nitrosation of
secondary amines and the diazotisation of primary
amines where the attack of N_2O_3 on the unprotonated
amine is rate determining.[20-23] Indeed it appears at
first that the pH–rate profile observed in our case
(Figure 4) might support such an interpretation. The
pH–rate profiles for the nitrosation of simple dialkyl-
amines for instance also exhibit maxima near pH 3.4.[21]
These maxima arise because the rate equation contains a
second-order dependence in nitrous acid. The linear
decrease in the concentration of unprotonated amine is
therefore counteracted in these cases by a quadratic
increase in the concentration of N_2O_3 as the hydrogen ion
concentration increases.

In contrast we have found that the nitrosodealkylation
rates exhibit first-order dependence in nitrous acid.
The rate maximum at pH 2.9—3.0 cannot therefore be
due to rate-determining initial nitrosation by N_2O_3.
Consequently we prefer the conclusion that the change
in the acidity profile at pH 3 indicates a change in the
rate-determining step. A similar situation has been
reported previously for the diazotisation of anilines in
concentrated aqueous perchloric acid.[24] Although we
cannot rule out that the initial nitrosation is rate limiting
at pH 3.0, we consider this possibility unlikely at pH
3.7—3.8, where nearly all our measurements with the
other amines were performed.

The measurements for different tertiary amines carried
out at pH 3.8 (Table) show that there are considerable
variations in the rates of nitrosodealkylation. The com-
parison of the pH-independent rate constants k'' and the
pK_a values for the amines indicates that no strict
correlation exists between the amine basicities and the
rate constants. The n-butyl derivative for example is
almost twice as reactive as the ethyl derivative although
it is the weaker base. In contrast, the rate constants
for the nitrosation of secondary amines by N_2O_3 correlate
with amine basicities,[25] as expected if electrophilic
nitrosation is rate determining. These results therefore
support the conclusion that the initial nitrosation step
is not rate limiting and consequently we prefer a
mechanism involving relatively rapid and reversible
nitrosation followed by slower subsequent steps leading

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to the products. With tertiary amines nitrosation results in the formation of nitrosoalkylammonium salts, species that cannot undergo irreversible tautomerisation or loss of a proton as commonly encountered with intermediates in the diazotisation of primary amines or the nitrosation of secondary dialkylamines. Thus the main difference to the nitrosation of primary and secondary amines is the absence of a rapid, irreversible product-forming step. Under such conditions the denitrosation of the nitrosotrialkylammonium ion can not be neglected, and it is necessary to assume that the initial nitrosation is reversible. The frequently acid-catalysed denitrosation of nitrosamines has been established by a considerable body of chemical experience as well as by recent kinetic measurements.[26] Our conclusions are summarised in the Scheme.

$$HNO_2 + HX \overset{K_1}{\rightleftharpoons} ONX + H_2O$$

$$HX \overset{K_2}{\rightleftharpoons} H^+ + X^-$$

$$R_3N + ONX \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R_3NNO^+ + X^-$$
$$\text{(1)}$$

$$R_3NNO^+ \overset{k_4}{\longrightarrow} R_2\overset{+}{N}=CHR' + HNO$$
$$\text{(1)} \qquad\qquad \text{(2)}$$

$$R_2\overset{+}{N}=CHR' \overset{k_5}{\longrightarrow} \text{products}$$
$$\qquad\qquad\qquad \text{(R}_2\text{NNO and R'CHO)}$$

$$X^- = OAc^-, NO_2^-, Cl^-, SCN^-, \text{and } H_2O$$

SCHEME
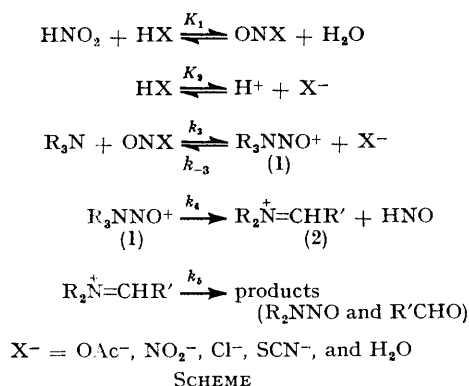
Application of the steady state principle to the nitrosoammonium intermediate (1) and the assumption that $k_5 \gg k_4$ lead to equation (5) for the rate of product

$$\text{Rate} = K_1 \frac{k_3 k_4 [H^+][R_3N][HNO_2][HX]}{k_{-3}K_2[HX] + k_4[H^+]} \qquad (5)$$

formation. With the assumption that $k_{-3}K_2[HX] \gg k_4[H^+]$ equation (5) can be simplified to give (6).

$$\text{Rate} = (K_1 K_3 k_4/K_2)[H^+][R_3N][HNO_2] \qquad (6)$$

Equation (6) reflects the dependence of the measured rates on the concentration of reagents correctly as shown by comparison with (4). We conclude therefore that the Scheme is consistent with the experimental results.

It is noteworthy that no firm conclusions can be drawn with respect to the nature of the active nitrosating species. The observed kinetic order of one with respect to nitrous acid in particular does not eliminate the possibility that the effective reagent is N_2O_3. On the other hand nitrosyl acetate is a likely alternative to N_2O_3 given that the acetic acid concentrations used in this work were high. The negligible effect of added chloride and thiocyanate is explicable if the rates of the nitrosation and denitrosation step are affected equally or if there is little competition of these ions with the excess of acetate and nitrite under the conditions of the reaction. Whilst the formation of nitrosyl chloride may be unlikely in this system, there was clear evidence for the presence

of nitrosyl thiocyanate when SCN⁻ was added. The absence of a marked catalytic effect of this ion may therefore be taken as additional support for the Scheme. This behaviour contrasts with the nitrosation of secondary amines where the addition of thiocyanate leads to considerable increases in the rate of nitrosation when the denitrosation reaction is unimportant.

The activation energy of 85 kJ mol⁻¹ observed for the nitrosodealkylation of triethylamine in this work is within the range expected for typical elimination reactions [27] but does not differ much from the values observed for normal N-nitrosation reactions.[21-23,28] Typical cyclic syn-(E_i) elimination reactions show activation energies which are somewhat higher. Since the substrate (1) is charged, however, significant medium effects will be encountered and an E_i mechanism for the elimination step (1) ⟶ (2) cannot be ruled out.

It is of interest to consider briefly the results of previous studies in the light of our conclusions. Smith and Loeppky contended on the basis of relative cleavage ratios of unsymmetrical amines and product analyses rather than rate measurements that the susceptibility of a tertiary amine to nitrosative cleavage is markedly reduced by base-weakening effects.[2] Wegler and Frank, on the other hand, observed that the ease of cleavage increases in the order cyclic < alkyl < benzyl.[29] Since benzylamines are weaker bases than their alkyl analogues these results seem to be in conflict. It must be remembered, however, that Wegler's observations have to be judged against the pH-dependent rate constants k, in contrast to Smith's which need to be compared with the true pH independent rate constants k''. The fact that there is, however, no correlation of the k'' values with the basicities among the strong amines does indicate that both structural, in particular steric effects, as well as electronic effects in the substrate play an important role in the rate-determining elimination step.

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Exhibit 32

山东华鲁恒升化工股份有限公司

SHANDONG HUALU-HENGSHENG CHEMICAL CO., LTD.

CERTIFICATE OF ANALYSIS

COMMODITY:N,N-DIMETHYL FORMAMIDE

BATCH NO.:20101026

THE RESULTS OF INSPECTION ARE AS FOLLOWS:

ITEM	SPECIFICATION	RESULTS
Pt-Co chrominance	≤10	5
Distillation test 0° C 101325Pa 151-155° C distillate volume	≥98.5	98.6
dimethylamine ppm	≤15	1
Formic acid ppm	≤25	2
PH:25° C 20%Aqueous solution	7.0-7.5	7.05
Electric Conductivity: 25° C 20%Aqueous solution, us	≤10	1.1
Moisture ppm	≤500	122
Fe ppm	≤0.05	0.002
Refractivity: $n_d^{25°c}$	1.4270-1.4290	1.4286
Methanel ppm	≤20	2
Heavy compoment (dimethyl Acetamide) ppm	≤500	43
DMF %	≥99.9	99.98

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Exhibit 33

Zhejiang Jianye Chemical Co.,Ltd

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Tel:+86-571-64149210，64149273   Fax:+86-571-64141300

URL: http://www.chinaorganicchem.com

E-mail: trade@chinaorganicchem.com   sale@chinaorganicchem.com

CERTIFICATE OF ANALYSIS

DATE:NOV.25,2012

LOT NO.:201209-207-80

Triethylamine Analysis

Property		Specifications	Results
Triethylamine,	wt%	99.5 Min	99.9
Water,	wt%	0.1 Max	0.02
Monoethylamine,	wt%	0.1Max	0.01
Diethylamine,	wt%	0.1Max	0.01
Ethanol,	wt%	0.1Max	N.D
Color ,	APHA	15 Max	10

浙江建业化工股份有限公司
ZHEJIANG JIANYE CHEMICAL CO., LTD.

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Exhibit 35

Article

Unveiling the Influence of pH on the Crystallization of Hybrid Perovskites, Delivering Low Voltage Loss Photovoltaics
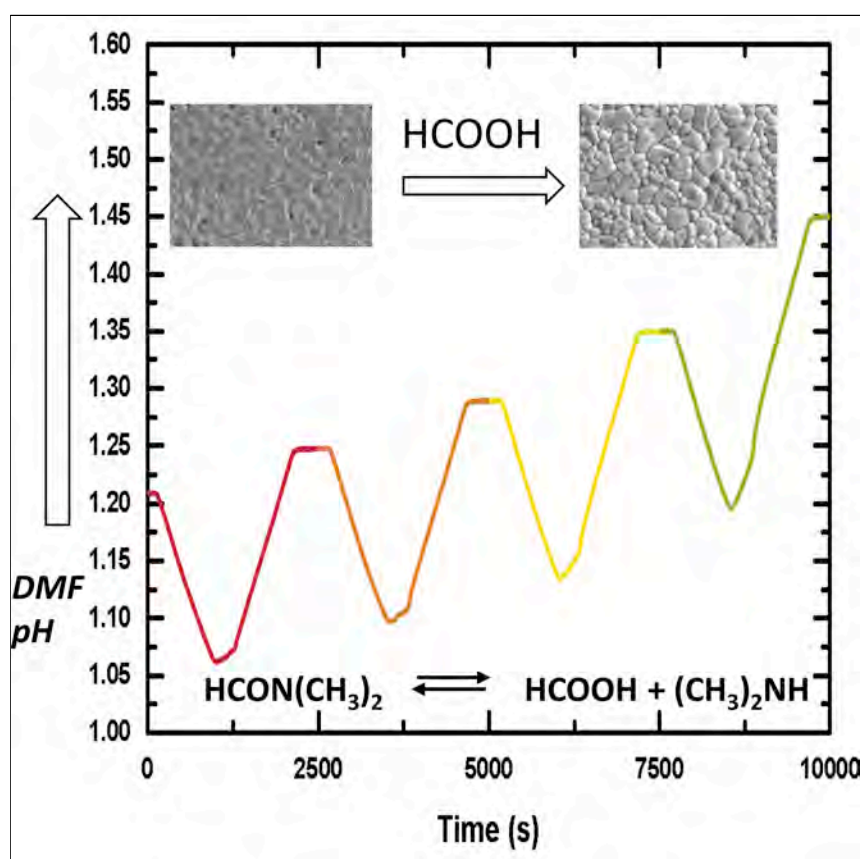
The degradation of *N*,*N*-dimethylformamide results in the formation of formic acid and dimethylamine. The changes in pH that occur as a result of this solvent degradation can be correlated to changes in the colloid concentration in perovskite precursor solutions. By tuning the pH and hence colloid concentration of these solutions, we improve the crystallization and optoelectronic quality of the perovskite films, resulting in solar cells with a record low loss in potential from bandgap to V_{OC} of 360 mV.

Nakita K. Noel, Martina Congiu, Alexandra J. Ramadan, ..., Michael B. Johnston, Bernard Wenger, Henry J. Snaith

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HIGHLIGHTS

Adding acid to DMF catalyzes its degradation producing formic acid and dimethylamine

Dimethylamine reduces the colloid concentration in DMF-based perovskite solutions

Reduction of colloid concentration yields improved optoelectronic quality

Record low voltage loss of 360 mV for a 1.57 eV bandgap material

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Article

Unveiling the Influence of pH on the Crystallization of Hybrid Perovskites, Delivering Low Voltage Loss Photovoltaics

Nakita K. Noel,[1] Martina Congiu,[1,2] Alexandra J. Ramadan,[1] Sarah Fearn,[3] David P. McMeekin,[1] Jay B. Patel,[1] Michael B. Johnston,[1] Bernard Wenger,[1,*] and Henry J. Snaith[1,4,*]

SUMMARY

Impressive power conversion efficiencies coupled with the relative ease of fabrication have made perovskite solar cells a front runner for next-generation photovoltaics. Although perovskite films and optoelectronic devices have been widely studied, relatively little is known about the chemistry of the precursor solutions. Here, we present a study on the hydrolysis of N,N-dimethylformamide, correlating how pH changes related to its degradation affect the crystallization of $MAPbI_{3-x}Cl_x$ perovskite films. By careful manipulation of the pH, and the resulting colloid distribution in precursor solutions, we fabricate perovskite films with greatly improved crystallinity, which when incorporated into photovoltaic devices reproducibly yield efficiencies of over 18%. Extending this method to the mixed cation, mixed halide perovskite $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$, we obtain power conversion efficiencies of up to 19.9% and open-circuit voltages of 1.21 V for a material with a bandgap of 1.57 eV, achieving the lowest yet reported loss in potential from bandgap to a V_{OC} of only 360 mV.

INTRODUCTION

During the last 5 years, lead halide perovskites have become a major focal point of the research community. These materials have shown applications in both light-emitting diodes[1,2] and lasers;[2] but most notably, they have leapt to the forefront of emerging photovoltaic technologies, achieving certified power conversion efficiencies (PCE) of up to 22.1%.[3] Lead halide perovskite materials have been shown to be efficient absorbers[4] but are also capable of generating free carriers and transporting charge.[5–7] This has allowed the exploitation of this material in various solar cell architectures, from mesoscopic to planar heterojunction devices.[8]

One of the main selling points of this material is the wide range of methods through which high-quality, crystalline films can be produced. These include a variety of solution deposition processes,[4,5,9,10] vapor deposition,[11,12] and mixtures of the two.[10] To date, solution deposition methods, specifically spin coating from either N,N-dimethylformamide (DMF) for one-step spin coating, or mixtures of DMF and DMSO for antisolvent quenching, remain the most widely used deposition methods for the production of lab-scale perovskite devices. Through careful fine-tuning of the specific perovskite composition and deposition methods, as well as the manipulation of the solvent through the use of compound solvents and solvent mixtures,[13–16] the PCEs of perovskite-based photovoltaics have soared. However, most efforts to increase the PCE have relied upon empirical processing parameter optimization, and relatively little attention has been paid to understanding the

Context & Scale

Metal halide perovskites have shown tremendous promise in optoelectronic devices and are of particular interest as absorber materials in solar cells, having achieved remarkable power conversion efficiencies in a staggeringly short period of time. Although improvements in deposition techniques have greatly increased the quality of perovskite films and have allowed perovskite solar cells to dominate the class of emerging photovoltaic technologies, relatively little focus has been placed on understanding the chemistry of the precursor solutions. Here, we elucidate how the hydrolysis and thermal decomposition of N,N-dimethylformamide, the most commonly used solvent for perovskites, has far-reaching effects on the crystallization and optoelectronic properties of perovskite films and show how controlling the degradation of this solvent allows us to achieve record low voltage losses in highly efficient perovskite solar cells.

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chemistry of the precursor solution, and how and why this affects the crystallization of perovskite films.

There have been numerous reports of the introduction of additives to precursor solutions as a means of influencing the crystallization and optoelectronic properties of perovskite films. Such additives include various metal cations,[17,18] halide and phosphorous acids,[19–22] ionic liquids,[23] organic small molecules,[24] and even water.[25] In all cases, the inclusion of small concentrations of these materials in the precursor solution increases the surface coverage, improves the crystallinity of the perovskite films, and also enhances the optoelectronic properties of the material. In particular, acids are widely used as additives to the perovskite precursor solution. We have previously shown a number of different ways in which acids can influence the crystallization and properties of the perovskite films; we have observed an increase in film quality upon addition of hypophosphoric acid (HPA) to a lead acetate-based methylammonium lead iodide (MAPbI$_3$) precursor solution,[20] and we have also employed hydroiodic acid (HI) to improve the film morphology of formamidinium lead iodide (FAPbI$_3$)[26] and reduce the crystallization temperature of CsPbI$_3$.[19] More recently, by using a mixture of HI and hydrobromic acid (HBr), combined with solution aging, we have demonstrated a method of controlling the crystallization kinetics of formamidinium-cesium mixed cation, mixed halide perovskite, via manipulation of the colloidal concentration in the precursor solution.[27] There have, however, been differing reports as to the optimal concentration of acid to add to perovskite precursor solutions,[19,21,26,28] as well as concerns as to how the addition of excess halide affects the overall perovskite stoichiometry and thus, its optoelectronic properties.[29]

One of the most important factors in the solution-based crystallization of any material is the solubility of the precursor salts in the chosen solvent. In the case of the salts required for perovskites, this solvent is typically DMF or a combination of DMF and DMSO. In its anhydrous state, DMF is a relatively neutral solvent with a pH of 6.3, which, upon exposure to water, undergoes hydrolysis to produce formic acid (FAH) and dimethylamine (DMA).[30–32] When DMF has been hydrolyzed and is in equilibrium with its degradation products, the solvent becomes basic, having a pH of approximately 11.[33] We have previously observed, in the growth of single crystals of the MAPbI$_3$ and MAPbBr$_3$ perovskites, that the time and temperature required for the mother liquor to reach supersaturation and produce crystals is dependent upon the age and consequently the pH of the solvent.[34] In this case, when using anhydrous DMF, the addition of formic acid was seen to decrease both the time and the temperature at which crystallization of the perovskite occurred. Through the addition of formic acid to anhydrous DMF, we can control the pH of the solvent, and as such, the solubility of the precursor salts. We postulate that by applying this methodology to the precursor solution for thin-film fabrication, we can have more precise control over the crystallization of perovskite films, and as such, greater reproducibility in device performance.

Here, we carry out a spectrophotometric investigation of the changes in pH of both neat and acidified DMF as it undergoes hydrolysis. We show how these changes in pH affect the ability of the solvent to solvate the perovskite precursor salts, and what effect this has on the resultant perovskite films. By utilizing formic acid to artificially tune the age of the DMF, we can control the crystallization of CH$_3$NH$_3$PbI$_{3-x}$Cl$_x$ films, to obtain reflective, pinhole-free films. Upon incorporation of these films into devices, we show enhanced PCEs of over 18%, and a significant decrease in the SD of the PCE. To investigate whether there is an added advantage to utilizing

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this approach in a slightly more controlled, fast crystallization process, or with a different perovskite composition, we apply this method to the widely used antisolvent quenching process. Here, through the addition of formic acid to a precursor solution of the mixed cation, mixed halide perovskite $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$, we see a marked increase in the photoluminescence quantum efficiencies (PLQE) of the films produced. Incorporating these films into photovoltaic devices yields stabilized efficiencies of up to 19.9% and open-circuit voltages of up to 1.21 V, showing a remarkably low loss in potential from bandgap to V_{OC} of just 360 mV, which is an improvement of 30 mV with respect to the previous lowest reported loss in potential for a lead halide perovskite solar cell.[35]

RESULTS AND DISCUSSION

The pH of a solution can be determined via two main pathways, through spectrophotometric or potentiometric analysis. The potentiometric determination of the pH of a solution is often used in cases where the solution under investigation is ion rich. Here, however, where our solution is in an organic solvent, which is ion deficient, the results obtained through this method of analysis may be confounded by diffusion of the ions from the reference electrode into the test solution, potentially introducing significant ambiguity. With this in mind, we have used spectrophotometric analysis to investigate any changes in the pH of DMF due to either hydrolysis or the addition of acids to the solvent.

For these experiments we use thymol blue as a pH indicator in DMF. Under strong acidic conditions, this indicator has a strong red/pink color (absorption peak at 550 nm), which transitions to yellow (absorption peak at 402 nm) and then blue (absorption peak at 615 nm) in neutral to slightly alkaline and highly alkaline environments, respectively.[36] We show the chemical structures of thymol blue in different pH regimes in Figure 1, along with the absorption spectra obtained in acidified, neutral, and alkalinized solutions of DMF.

In Figure 1F we show the absorption of thymol blue in a neutral environment, where we observe that the most prominent absorption peak is at 402 nm. This peak is most visible under neutral or weakly basic conditions. In order to speed up the rate of hydrolysis of DMF, we subject the solvent to temperature cycling, gradually increasing the temperature from 25°C to 95°C, followed by gradually cooling the solution back down to 25°C over a period of 45 min. As aforementioned, when DMF is hydrolyzed, the pH of the solution moves toward a more basic regime. As such, we expect that as this occurs, we should see a decrease in the absorption peak at 550 nm and a subsequent increase in the peak at 402 nm (see Figure 1A). Thus, to investigate the pH change that occurs as a consequence of this hydrolysis, we measure the change in the absorbance at 402 nm as a function of time and temperature. We present the results in Figure 2. In the first step of the temperature cycle, the absorbance remains constant as the solution is held at 25°C for 2 min. The second step of the cycle involves a gradual increase in temperature over approximately 15 min to 95°C, during which we see a decrease in the absorbance at 402 nm. This decrease in the absorbance indicates that the solution becomes less basic. The chemical reaction for the hydrolysis of DMF is described by Equation 1:

$$HCON(CH_3)_2 \overset{H_2O}{\rightleftharpoons} HCOOH + (CH_3)_2NH . \qquad \text{(Equation 1)}$$
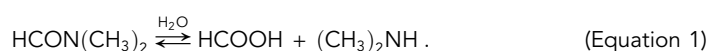
With increasing temperature, the equilibrium position of the above reaction shifts to the right, thus producing more formic acid and dimethylamine. As discussed by Nayak, Moore, and co-workers, when considering the influence of temperature

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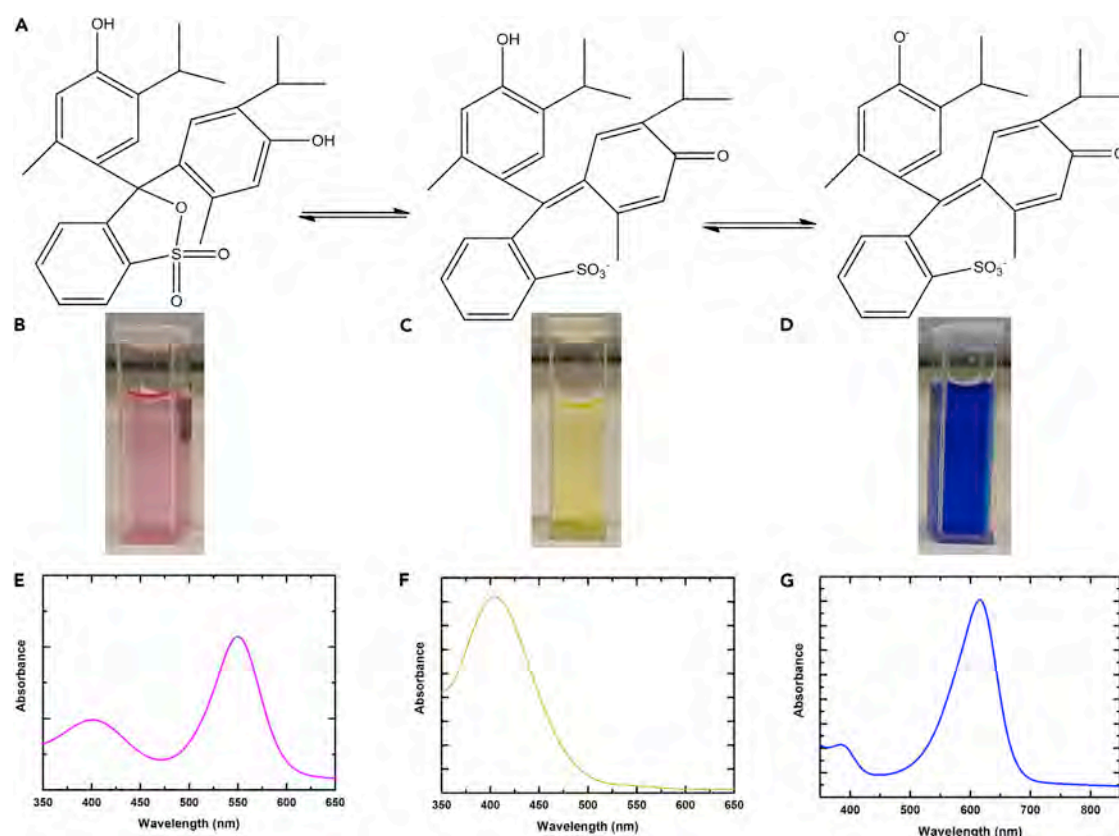
Figure 1. Chemical Structure and Absorption Spectra of Thymol Blue

(A) Chemical structures of thymol blue in (from left to right) acidic, neutral, and basic environments.
(B–D) Photographs of thymol blue in (B) acidified DMF, (C) neat DMF, and (D) alkalinized DMF.
(E) Absorption spectra of the solutions shown in (B).
(F) Absorption spectra of the solutions shown in (C).
(G) Absorption spectra of the solutions shown in (D).[11]

upon the crystallization of large single crystals from solution, we expect that the temperature increase will also encourage the dissociation of the formic acid, thus decreasing the basicity of the DMF solution.[34] In the third step of the temperature cycle, the DMF is held at 95°C for 5 min. At this point, we see that the solution very slowly begins to recover, experiencing a small increase in basicity. The final step of the temperature cycle involves a gradual return to room temperature, during which we see the solution become increasingly basic, even more so than before the temperature cycling. We can interpret these results to indicate that while increasing the temperature results in an increase in acidity (or decrease in basicity) due to the reversible dissociation of formic acid; subsequent to the heating cycle, the DMF becomes more basic than in its anhydrous state, most likely due to hydrolysis having occurred. This is consistent with the formation of dimethylamine, which has a lower pK_a ($pK_a^{conj.\ acid}$ = 10.4 in DMF) than that of formic acid (pK_a = 18.9 in DMF).[37] This means that dimethylamine is a stronger base than formic acid is an acid, and thus, when they are both present in similar concentrations, dimethylamine would have a larger influence on the pH of a solution. In Figure 2B, we show the progressive changes in the 402 nm absorbance of thymol blue in DMF for four consecutive heating cycles. The solution was left to rest at 25°C for 1 hr between each heating cycle. Here, we see that the increase in basicity that occurs after temperature cycling

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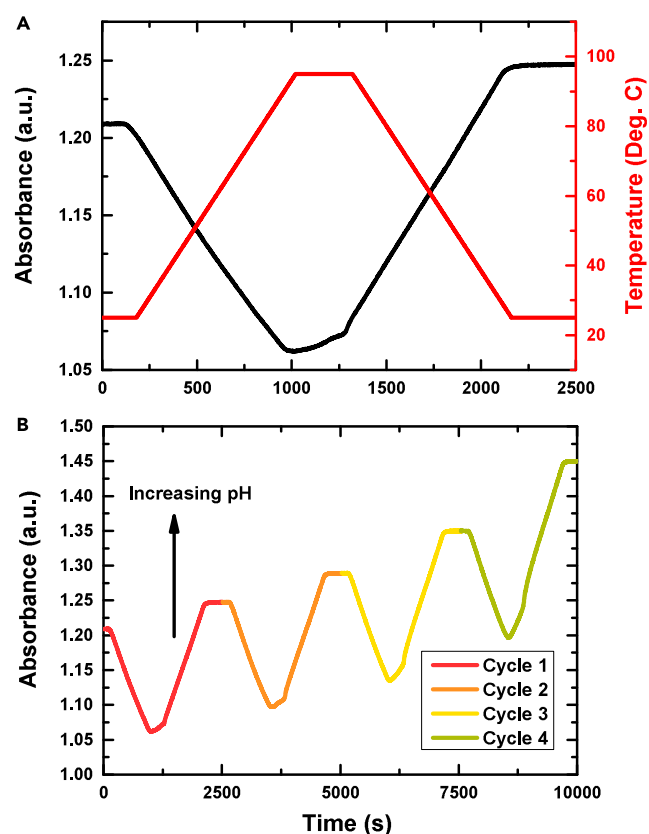
Figure 2. Temperature Cycling of DMF

(A) Evolution in the absorbance of thymol blue in DMF at 402 nm with time and temperature. The change in absorbance is depicted by the solid black line and the corresponding temperature curve is shown by the dashed red line.

(B) The change in 402 nm absorbance of thymol blue in DMF over four successive temperature cycling events. Note that the higher absorbance on each cycle corresponds to the lower temperature of 25°C.

is irreversible. Even when the solution is left to rest, it does not appear to recover, with the pH of the solution at the beginning of the second cycle being exactly the same as it was at the end of the first cycle.

This trend continues for all subsequent temperature cycles, with the change in absorbance, and thus pH, becoming greater as the cycling continues. Another interesting feature of these temperature-dependent measurements is how the solution behaves when held at high temperature. We see the increase in absorbance that occurs when the solution is held at 95°C becoming steeper with each cycle. During the course of this measurement, we expect that the decrease in absorbance is due to the deprotonation of formic acid with an increase in temperature. However, the gradual increase in absorbance that we see when the solution is held at high temperature is likely due to the fact that with each temperature cycle, the hydrolysis of DMF is being pushed closer and closer to its equilibrium point. This will create an increasingly more basic solution, which when held at a constant high temperature, proceeds more quickly to its equilibrium point. Indeed, if we hold the solution at high temperature over a period of hours, we see the absorbance continues to increase until it reaches a plateau, which we attribute to the hydrolysis reaching equilibrium conditions (see Figure S1).

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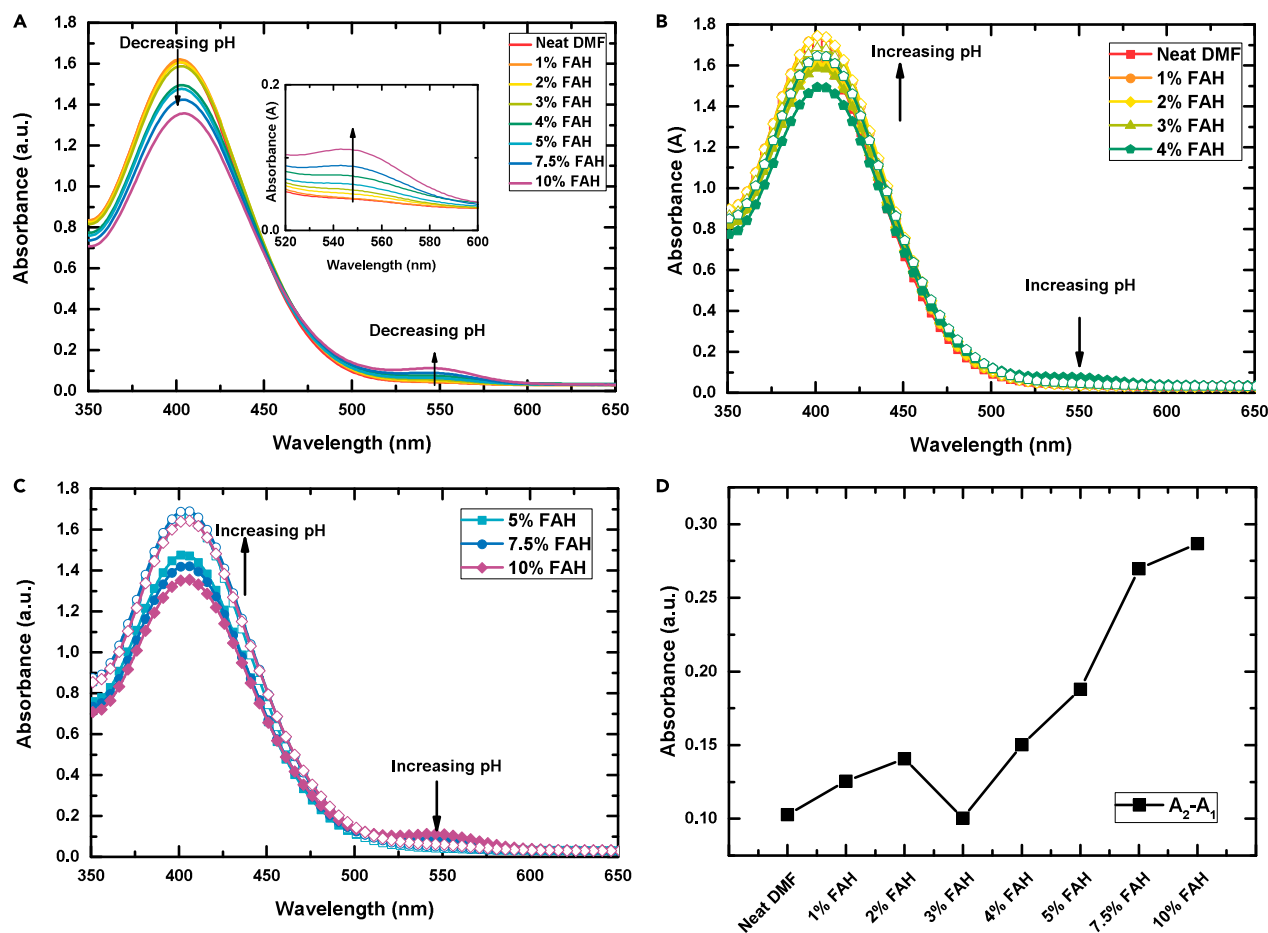
Figure 3. Addition of Formic Acid to DMF

(A) Absorption spectra of thymol blue in DMF with the addition of increasing amounts of formic acid (FAH). A close up of the changes in the 550 nm absorption peak is shown in the inset.

(B and C) Absorption spectra of thymol blue in acidified DMF before (B) and after (C) temperature cycling. Solid symbols show the absorption before cycling and open symbols depict the spectra after temperature cycling.

(D) Change in the value of the 402 nm absorption peak before and after temperature cycling.

We now consider the effect of acidic additives on the pH of DMF. There have been various reports of the use of acids as an additive to the perovskite precursor solution, all showing improved crystallization, as measured by increased surface coverage of the perovskite films on the substrates and enhanced device performance in solar cells. Here, we use HCl, HPA, and formic acid to determine what, if any, effect they have on the pH of DMF. While HI is the most commonly used acid additive for $MAPbI_3$ solutions, it has recently been shown that it can result in slight shifts in the bandgap of the material.[29] For this measurement, we have not investigated HI as an additive due to the fact that the triiodide absorption peak overlaps with the 402 nm absorption peak of thymol blue, making it difficult to disentangle pH effects from the oxidation of iodide ions. We show our results for the addition of formic acid to DMF in Figure 3.

In Figure 3A we see that, in neat DMF, the absorbance of thymol blue at 550 nm is negligible, but with the addition of increasing volumes of formic acid, we observe a decrease in the absorbance at 402 nm and a corresponding increase in the absorbance at 550 nm. This is expected to occur as the solution becomes increasingly

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acidic. Interestingly, however, we see that after temperature cycling these solutions (see Figures 3B and 3C), the higher the acid concentration used, the more basic the solution becomes. This is more clearly seen in Figure 3D where we show the difference in the 402 nm absorbance before (A_1) and after (A_2) temperature cycling. We observe a similar trend for the addition of the more common acidic additives such as HCl and HPA (see Figure S2). Strong acids such as the hydrohalic acids are very commonly used as catalysts in the hydrolysis of DMF.[38] The stark increase in the basicity of the solution with the addition of large amounts of acid can therefore be explained by an increase in the rate of hydrolysis of the DMF and the resultant increase in the concentration of dimethylamine present in the cycled DMF. The hydrolysis of DMF is typically a slow process, and an interesting point to note is that the simple addition of deionized water is not enough to produce such a rapid change in the pH of the DMF. This excludes the possibility that, on this timescale, these effects are simply due to the water content of the given acids (see Figure S3). Another point of note is that while the addition of formic acid, deionized water, and HCl to DMF results in very similar absorption profiles when subjected to temperature cycling, the addition of HPA appears to result in an almost immediate and continuous increase in basicity with temperature (see Figure S4).

We, and others, have previously shown that precursor solutions of perovskite materials contain colloidal dispersions, rather than being entirely composed of fully solvated ions.[27,34,39] Having shown here that the addition of acid changes the composition of DMF as a solvent and irreversibly changes the pH, we now investigate how this affects its ability to solvate the perovskite precursor salts. In order to do this, we prepare precursor solutions of $MAPbI_{3-x}Cl_x$ in DMF at 38 wt % (3 M MAI:1 M $PbCl_2$) and do dynamic light scattering (DLS) measurements on the neat solution, as well as on solutions with acidic additives. We present the results of these measurements in Figure 4. We note here that while the absolute pH of the full solution (DMF + salts) will not be exactly the same as for the neat and acidified DMF without salts, the important metric is the overall change or variation in the pH of the solution that results from using DMF in different stages of hydrolysis.

From the results shown in Figure 4, we see that in the neat precursor solution, without the addition of acid, there are some large (hundreds of nanometers) colloids, along with smaller colloids with diameters between 1 and 10 nm. It is important to note that the overall concentration of the perovskite precursor solution was kept constant for all experiments. For example, the neat solution was made such that 38 wt % of the precursor salts was dissolved in 1 mL of DMF. Similarly, for the 1% FAH solution, the same mass of salts was dissolved in 1 mL of solvent (990 μL DMF+ 10 μL FAH). With the introduction of 1 vol % FAH and 2 vol % FAH, we observe a successive decrease in the number of larger colloids present in the solution. Upon the addition of 3 vol % FAH, these larger colloids completely disappear, suggesting that at this point, we have very close to a true solution or at least a solution of solvated small complexes and ions. With a stronger acid such as HCl, the addition of 1 vol % of acid to the precursor solution is enough to entirely remove the presence of larger colloids. In the case of HPA, we see that the addition of just 0.2 vol % of HPA to the precursor solution completely rids the solution of the presence of large colloids. This coincides with the previous observations that the addition of 3–5 μL of HPA to 1 mL of a perovskite precursor solution was sufficient to result in a significant enhancement in film quality.[20]

We have previously shown the dissolution of colloids in perovskite precursor solutions with increasing temperature.[34] Here, we investigate the effects of

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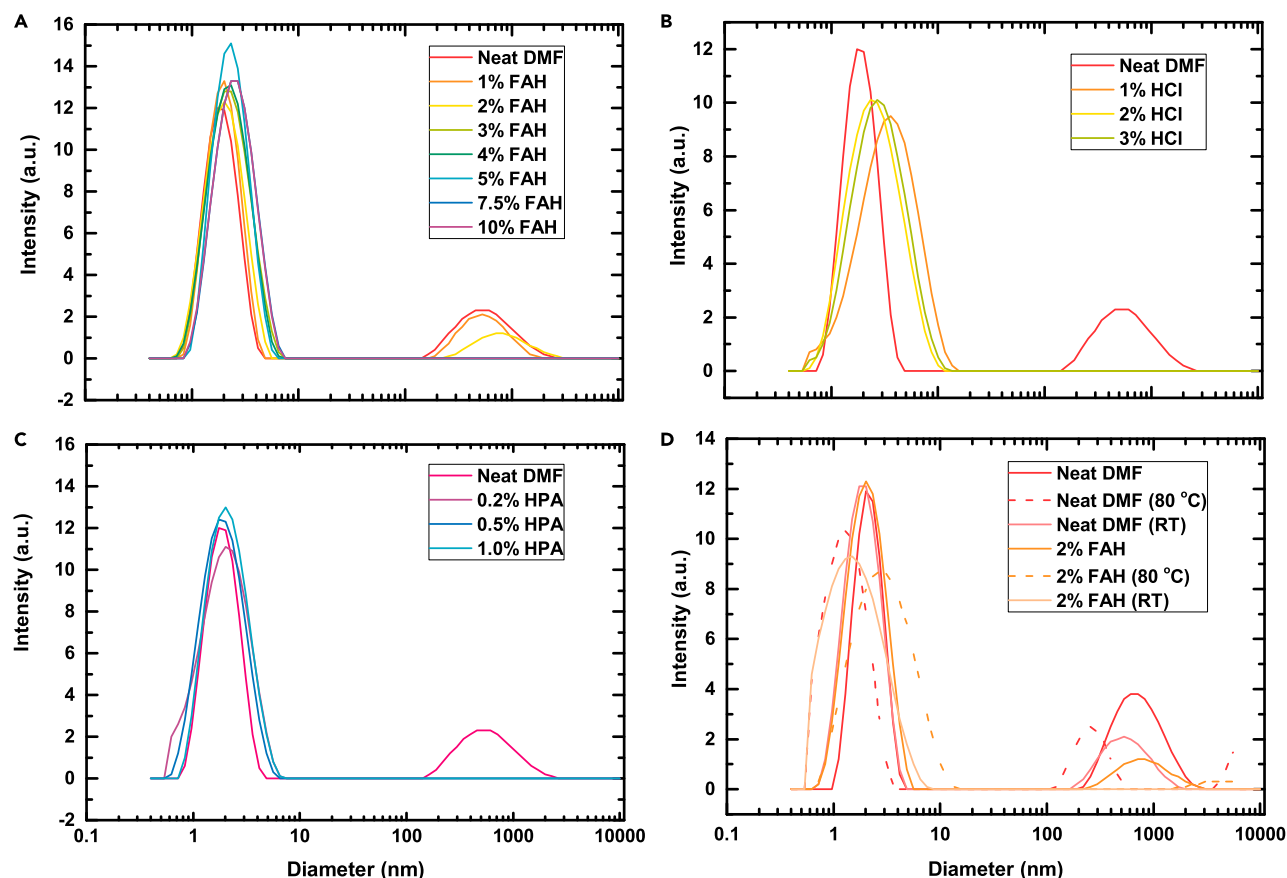
Figure 4. Dynamic Light Scattering Measurements

(A–C) Change in the size distribution of colloids present in a 38 wt % precursor solution of $MAPbI_{3-x}Cl_x$ with the addition of (A) formic acid (FAH), (B) HCl, and (C) HPA.

(D) Change in the size distribution of colloids with heat, and both heat and acid. Solid traces show the measurements taken before heating and after being heated and cooled down to room temperature (RT), while dashed lines show the colloidal distribution at 80°C

temperature on the presence of colloids in a neat precursor solution, as well as in a solution to which we have added formic acid. We initially measure the solution at 25°C, then heat the solution to 80°C, and re-measure it. Subsequently, we allow the solution to cool down to room temperature and then take a final set of measurements. We note that while there are both small and larger colloids present in the neat DMF solution, upon heating the solution up to 80°C, we observe that both the size and number of these colloids decrease. However, when the solution is cooled to room temperature, it returns to a point close to its original state, albeit with a smaller proportion of larger colloids. For the acidified solution, we chose to add 2 vol % FAH, as at this acid concentration there are still a few large colloids present in the solution. Here, when we heat the solution, we observe a similar trend as with the neat DMF solution: a significant decrease in the number of large colloids present in the solution. Interestingly, when we allow this acidified solution to cool down to room temperature, it does not return to its original state. Rather, we no longer detect the large, micrometer-sized colloids and observe that in addition the distribution of smaller colloids or complexes have shifted toward smaller diameters. This indicates that heating the solution in the presence of formic acid causes it to go through an irreversible change, which effectively allows the solvent to better solvate the precursor salts.

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We know from our previous work that heating a solution of perovskite precursor salts in DMSO causes the deprotonation of the methylammonium cation to form methylamine, which has been shown to be an excellent solvent for perovskite salts.[13,34,40] This process is likely the cause of the decrease in the number and distribution of colloids that we see in both the neat and acidic precursor solutions. However, since this process should be completely reversible upon cooling, these results suggest that there is another mechanism at play and points to the role of DMF as a solvent. We have seen from the temperature cycling measurements shown in Figures 1 and 2 that as DMF is hydrolyzed, it becomes increasingly basic due to the formation of dimethylamine. The rate of hydrolysis of DMF also appears to increase with the addition of acid.[31] We propose that the dimethylamine produced by the hydrolysis of DMF, as with other amines that we have previously investigated,[13] increases the solvating power of DMF, making it a better solvent for the perovskite precursors. To test this theory, we bubble dimethylamine into the precursor solution using a method that we have previously described for bubbling methylamine into solvents.[13] We see that for time intervals as short as 1 min, there is a large reduction in the size and number of colloids that are present in the precursor solution, consistent with our hypothesis that the generation of dimethylamine in hydrolyzed DMF is the most likely cause of further colloid dissolution (see Figure S5).

Controlling the distribution of colloids in a perovskite precursor solution has been shown to be extremely important to the morphology of the final film.[27,39] In the present work, we have seen that the addition of formic acid to the perovskite precursor solution results in the dissolution of colloids. By starting with a concentration of formic acid for which all large colloids are dissolved (3% FAH), we are able to reproducibly obtain specular, pinhole-free films with larger, more highly oriented crystals. We present images of such films and X-ray diffraction data in Figure 5.

In Figure 5, we show top view SEM images and the corresponding diffractograms of $CH_3NH_3PbI_{3-x}Cl_x$ films formed on fluorine-doped tin oxide (FTO) substrates coated with a compact layer of SnO_2,[41] from precursor solutions made with neat and acidified DMF. While we observe a few pinholes present in the film that we processed from the neat solution, we observe no visible pinholes in films that we have processed from solutions containing formic acid. Furthermore, we begin to see more defined crystal domains. To assess the crystallinity of the films, we have performed X-ray diffraction (XRD) measurements on these films and find that the addition of formic acid results in a significant increase in the crystallographic texture of the film, as is demonstrated by the doubling of the intensity of the 110 peak. It is interesting to note that while there is almost a doubling of the intensity of the 110 peak when 3 vol % of formic acid is added to the solution, this increase does not continue linearly as more acid is added. We observe similar effects (i.e., increased surface coverage and increased crystallinity and/or orientation of the grains) for the addition of halide acids, but at different concentrations (see Figure S6). We note, however, that while we observe an increase in surface coverage for some concentrations of the halide acids, we do not completely eliminate pinholes in the films we have fabricated using halide acids. We show these results for different acids in Figure S7. It has recently been reported that aging the MAPbI₃ perovskite precursor solution at 70°C for 48 hr is beneficial for film formation, in that it allows the production of pinhole-free films with large crystalline domains.[42] We believe that this observation is largely due to the dissolution of colloids in the precursor solution, which occurs as a result of changes to the solvent. Both the application of heat and the addition of acids to DMF cause an accelerated aging of the solvent, causing the onset of degradation of DMF, which results in the production of dimethylamine.

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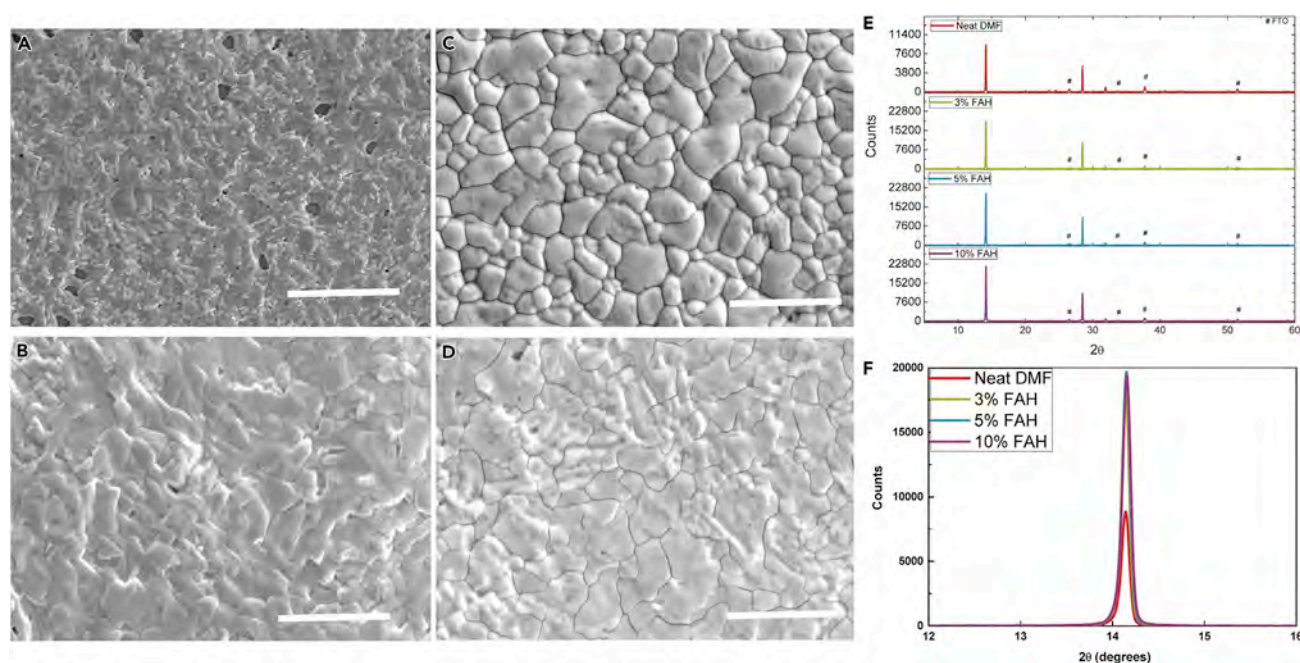
Figure 5. Scanning Electron Microscopy and X-Ray Diffraction

(A–D) Top view SEM images of $MAPbI_{3-x}Cl_x$ perovskite films coated from neat and acidified DMF solutions, with formic acid additions of (A) 0 vol %, (B) 3 vol %, (C) 5 vol %, and (D) 10 vol %, respectively. The scale bars on all images represent a distance of 5 μm.

(E) X-ray diffractograms of films from control and acid-modified solutions.

(F) Close-up view of the 110 peak of the perovskite films shown in (E).

Dimethylamine is produced through both the thermal degradation and the hydrolysis of DMF, allowing for, in both cases, the dissolution of colloids in the precursor solution. We propose that the "universal" improvements that have been seen in $MAPbI_3$ perovskite solar cells as a result of the addition of acids[19–21] and water to the precursor solution, and even through the popular deposition method of hot casting,[42–44] are due mainly to the dissolution of colloids, with the latter occurring as a result of changing the chemistry of the solvent and increasing its solvation power.

To ascertain the effects that using different acidic additives in the perovskite precursor solution have on the composition of the resultant film, we have carried out depth-profiling time-of-flight secondary ion mass spectrometry on films fabricated using equal volumes of the most common acid additives compared with formic acid. We present the results of these measurements in Figures S8–S10 and show that, while films formed using neat DMF and DMF + formic acid solutions are incredibly similar, films formed in the presence of other acid additives indicate the inclusion of phosphorus or excess halide in the films.

Having shown the effect of manipulating the pH of DMF on colloid concentration, and thus film quality, we proceed to investigate how this affects the operation when the perovskite films are incorporated into solar cells. In order to do this, we fabricate planar heterojunction solar cells using the device structure $FTO/SnO_2/MAPbI_{3-x}Cl_x/spiro$-OMeTAD/Ag. We present these results in Figure 6. In Figure 6A, we show statistics collected from eight different batches of experiments (the complete performance parameters are shown in Figure S11). Here, we see that the addition of formic acid results in a steady increase in the PCE, peaking with the addition

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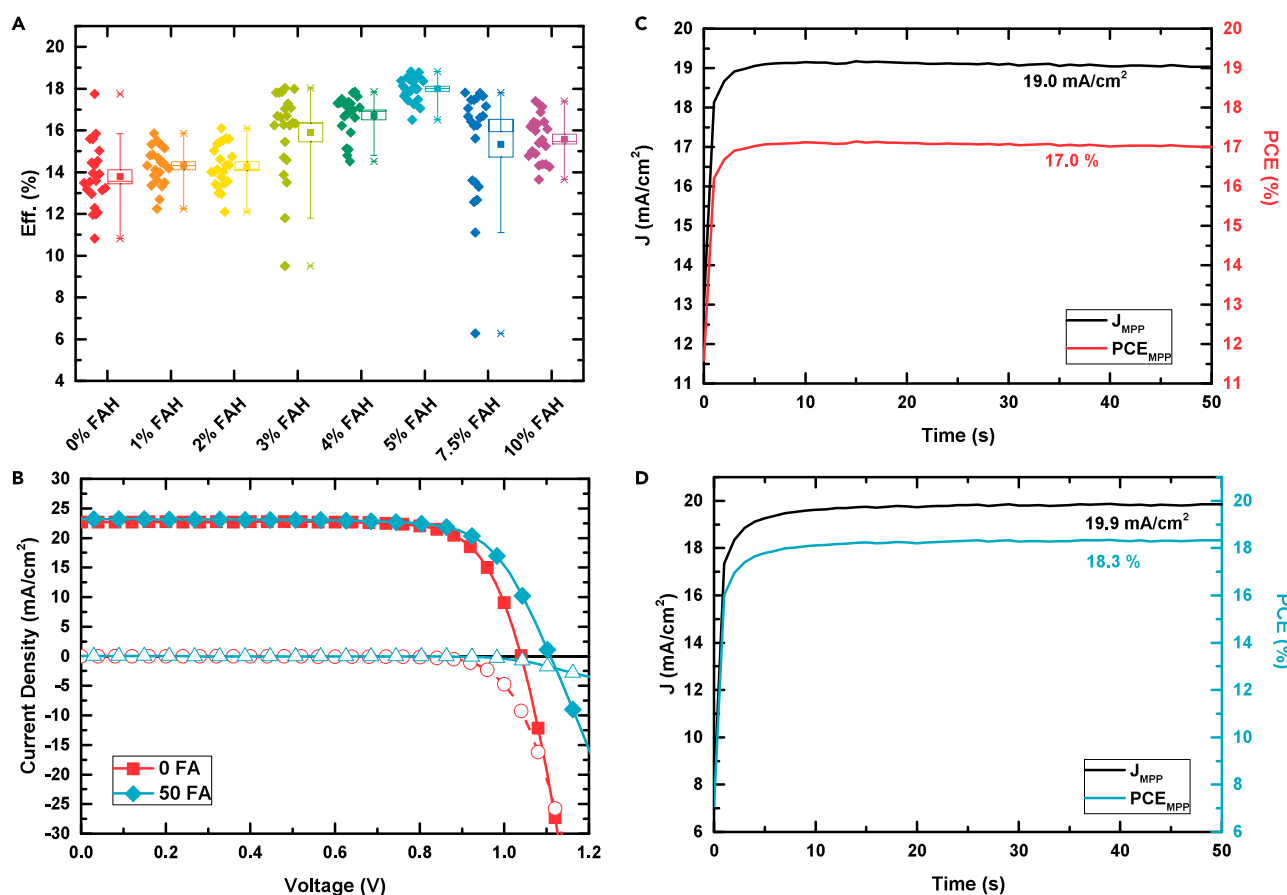
Figure 6. Current-Voltage Characteristics

(A) Statistics on the efficiency of eight individual batches of solar cells prepared using neat and acidified DMF, using from 0 to 10 vol % of formic acid as an additive to the precursor solution. Whiskers indicate data that fall within 1.5 IQR.

(B) Current-voltage curves of solar cells fabricated using neat DMF as a solvent, as well as DMF with 5 vol % of formic acid added. Solid symbols represent the light JV measurement, while hollow symbols show the dark JV.

(C and D) Stabilized current density (C) and PCE (D) of the devices shown in (B). These measurements are taken by holding the devices at their maximum power point over a period of 50 s.

of 5 vol % formic acid, after which there is a decrease in the mean efficiency. Notably, we observe the average PCE to increase from 14% to 18% with the addition of formic acid. We see a similar trend with the direct addition of dimethylamine (Figure S12). We give the performance parameters of the devices in Table 1. In Figure 6B, we show the current-voltage curves of the champion control device, fabricated with the neat solution, at an efficiency of 17.7%, as well as the champion device for the optimized concentration of formic acid, for which we measure a PCE of 18.8%. We note that while both of these devices show hysteresis in the current-voltage curves, the degree of hysteresis is reduced with the addition of the formic acid (see Figure S13). We present the steady-state maximum power point current density and PCE of these devices in Figures 6C and 6D, which also shows an increase from 17.0% to 18.3% with the addition of formic acid. The external quantum efficiency (EQE) of the champion devices is shown in Figure S14. Notably, in addition to a significant increase in the overall PCE, we also observe a much narrower distribution of performance for the cells with the optimum addition of formic acid, with an increase in the average efficiency and reduction in the SD from 13.8% $\pm$ 1.5% to 18.3% $\pm$ 0.5%. We also note that, as far as we are aware, this is the highest

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Table 1. Performance Parameters

[FAH]	J_{SC} (mA/cm^2)	η (%)	V_{OC} (V)	FF (a.u.)	η_{MPP} (%)
0	20.8 ± 1.4 (22.6)	13.8 ± 1.5 (17.7)	1.02 ± 0.04 (1.04)	0.64 ± 0.04 (0.74)	17.0
10	19.5 ± 1.1 (20.9)	14.3 ± 0.9 (15.9)	1.05 ± 0.08 (1.04)	0.69 ± 0.03 (0.72)	
20	20.0 ± 1.0 (21.5)	14.2 ± 1.0 (16.1)	1.06 ± 0.04 (1.07)	0.67 ± 0.03 (0.69)	
30	22.1 ± 1.0 (23.2)	15.9 ± 2.1 (18.0)	1.05 ± 0.03 (1.04)	0.68 ± 0.07 (0.75)	
40	22.7 ± 1.2 (23.8)	16.7 ± 1.0 (17.9)	1.06 ± 0.03 (1.05)	0.70 ± 0.01 (0.71)	
50	23.0 ± 0.5 (23.2)	18.3 ± 0.5 (18.8)	1.11 ± 0.02 (1.10)	0.72 ± 0.02 (0.74)	18.3
75	22.2 ± 1.5 (23.3)	15.3 ± 2.5 (17.8)	1.05 ± 0.02 (1.07)	0.65 ± 0.10 (0.72)	
100	21.0 ± 0.9 (22.0)	15.6 ± 1.1 (17.4)	1.07 ± 0.03 (1.10)	0.71 ± 0.03 (0.73)	

Performance metrics of devices made with and without formic acid. Average cell efficiencies are presented with the SD. Values for the champion devices of each acid concentration are given in parentheses.

steady-state efficiency reported to date for a planar heterojunction perovskite solar cell employing a perovskite layer that is spin-coated from a DMF-based solution via a simple one-step spin-coating process, without additional air drying or solvent quenching during coating.

To investigate if a similar effect can be obtained using both a different perovskite, as well as a fast crystallization approach, we apply this methodology to the antisolvent quenching fabrication process. Here, we vary the formic acid concentration in the DMF used to fabricate a precursor solution of the mixed halide, mixed cation perovskite $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$, films of which are most frequently fabricated through the solvent-quenching process, using a mixed solvent (4:1 v/v DMF:DMSO). As an initial test to see if there is any measurable effect on the optoelectronic properties of the films produced through this route, we perform PLQE measurements, which provide an indication of the extent of non-radiative recombination in the films. We present these results in Figure S15. We see from these measurements that the addition of formic acid to the solvent results in an increase in PLQE from ∼13% for the films fabricated without acid to a maximum of just over 20% for films cast from solutions containing 7.5 vol % FAH. With this processing route, the crystallization is rapid and largely controlled by the deposition of the antisolvent, hence we do not expect to see as significant changes in morphology or crystallinity as we observed with the one-step spin coating that we employed for the $MAPbI_{3-x}Cl_x$ perovskite. From SEM images (Figure S16), we see that in films both with and without formic acid, we have uniform coverage and very similar grain sizes. We show the XRD of these films in Figure S17 and show that the crystallinity of the films (as determined by the width and intensity of the 110 peak) is very similar, although the exact orientation of the crystals (as inferred from the relative intensity of the different XRD peaks) appears to be slightly different. Therefore, for this material and processing route, the main impact of the addition of formic acid appears to be reduction in the density of defects sites responsible for non-radiative recombination. Presumably these defect sites arise when films are crystallized from, or in the presence of large colloids. Having identified the optimal formic acid concentration from the PLQE measurements, we proceed to incorporate these films into complete planar heterojunction solar cells, with the same device structure as the cells that we presented previously. Through the addition of 7.5 vol % of acid to the precursor solution, we are able to obtain a maximum PCE of 19.9%. We present the current-voltage curves and stabilized efficiencies in Figure 7. As with the $MAPbI_{3-x}Cl_x$ perovskite devices that we presented earlier, while $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ devices fabricated from both the neat and acidified precursor solutions exhibit some hysteresis, we observe a reduction in the

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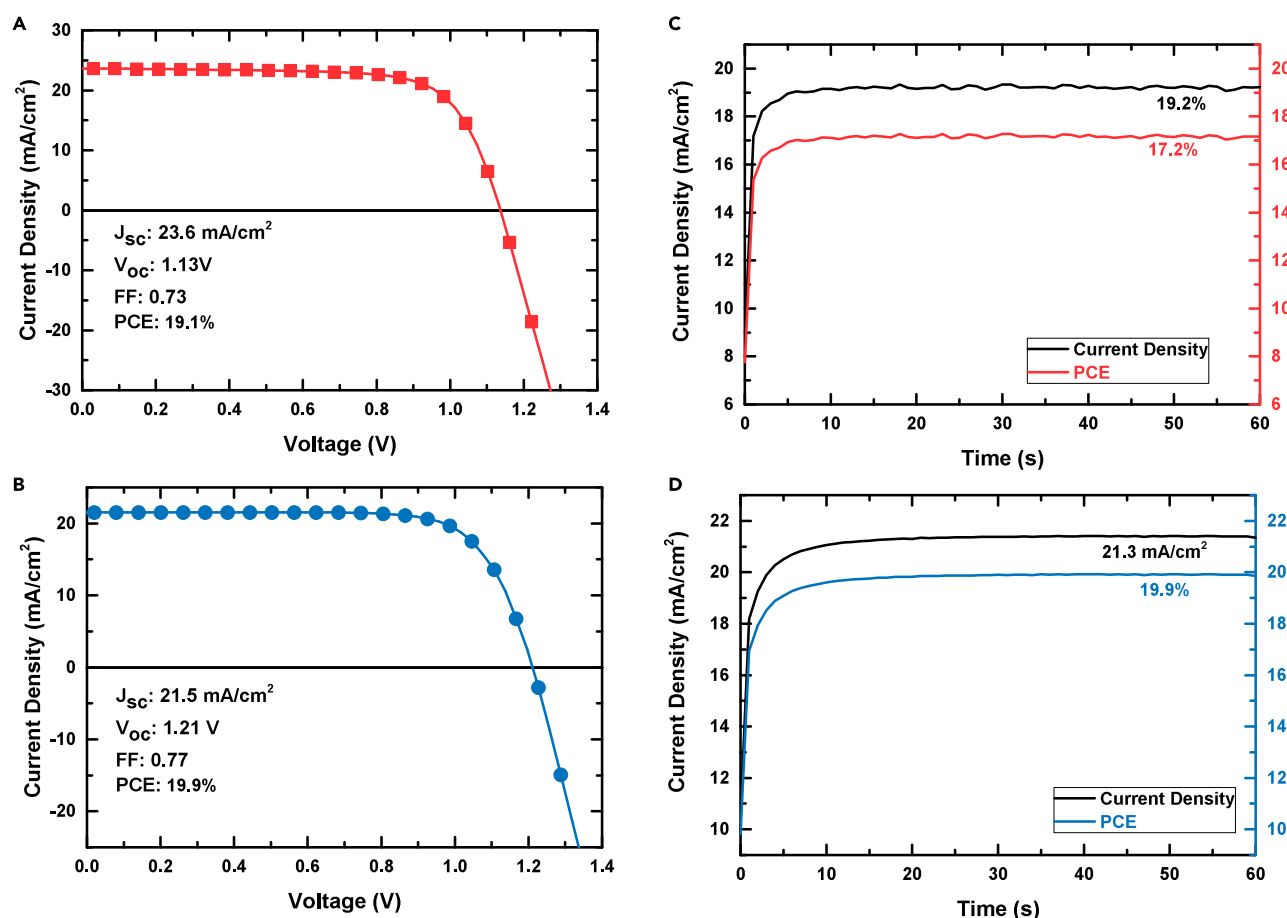
Figure 7. Current-Voltage Characteristics

(A–D) Current-voltage curves of $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ perovskite solar cells fabricated from (A) a neat precursor solution and (B) an acidified precursor solution. The corresponding stabilized currents and efficiencies are shown in (C) and (D).

hysteresis with the addition of formic acid (forward and reverse scans are shown in Figure S18).

Here, we observe that much like in the case of the $MAPbI_{3-x}Cl_x$ perovskite cells, we obtain a noticeable increase in the open circuit with the addition of formic acid. We are able to increase the open-circuit voltage in these perovskite devices by 70–80 mV. To confirm that these gains in open-circuit voltage are indeed due to improvements in the quality of the perovskite layer and not to an incidental change in the underlying SnO_2 layer, we have performed experiments in which the SnO_2 layer is pre-treated with a DMF/FAH mixture and dried at 100°C for 10 min before the deposition of the perovskite layers. We show the results of these experiments in Figures S19 and S20. At low formic acid concentrations, we see very little to no effect on the voltage of the devices. However, at higher concentrations, we see a decrease in the voltage, suggesting that the increases that we observe in our devices are indeed due to improvements in the quality of the perovskite layers deposited. In the case of the $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$, which has a bandgap of approximately 1.57 eV, as determined from a Tauc plot (See Figure S21), we obtain the lowest yet reported loss in potential from bandgap to V_{OC} of only 360 mV. To the best of our knowledge, the lowest previously reported loss in potential reported for lead halide perovskite solar cells is 390 mV.[35] We show the EQE for this device

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in Figure S22. This, in combination with the PLQE results, indicates that there is a significant reduction in the non-radiative recombination taking place both within the perovskite films and in complete devices when the films are processed from solution containing a small quantity of formic acid. We note, however, that for this precise methodology, we observe a significant reduction in the short-circuit current density upon formic acid addition. This may in part be due to a significant reduction in film thickness even though we have retained a constant overall concentration of the precursor solution. While the exact cause of this phenomenon is under investigation, we postulate that this is in part due to a reduction in the concentration of colloids in the solution. Nevertheless, through these results, we show that the role of solvent pH in the crystallization of perovskite films is crucial not only to one-step, slow crystallization methods but also the widely used solvent-quenching method; and importantly, illuminates a path to achieving efficient, low voltage loss perovskite photovoltaics.

Conclusions

In summary, by using thymol blue as an indicator, we show through spectrophotometric measurements how the pH of DMF changes over time. In addition, we reveal that commonly used acidic additives such as hydrohalic acids and HPA increase the rate of hydrolysis of DMF, causing the production of dimethylamine and delivering an unanticipated increase in the basicity of the solution. Through performing DLS measurements on perovskite precursor solutions made with both neat and acidified DMF, we observe that the addition of acids (HCl, HI, HPA, and formic acid) results in irreversible changes in DMF, as well as the dissolution of colloids in the perovskite precursor solution. We postulate that dimethylamine, which is produced as a result of the thermal degradation and/or hydrolysis of DMF, plays an important role in the dissolution of colloids, which in turn results in enhanced film quality. By introducing formic acid into the perovskite precursor solution, we are able to artificially age the DMF solution without incorporating excess halide or phosphorus into the films, thereby eliminating another potential variable in perovskite processing. By adding an optimal concentration of formic acid to perovskite solutions made using anhydrous DMF, we are able to reproducibly obtain device efficiencies of over 18% for the $MAPbI_{3-x}Cl_x$ perovskite and approach steady-state efficiencies of close to 20% with the mixed cation, mixed halide perovskite $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$. Critically, for solar cells fabricated from this perovskite composition, we achieve open-circuit voltages of up to 1.21 V with an absorber bandgap of only 1.57 eV. The effects of DMF degradation on the quality and reproducibility of perovskite-based devices have been largely unexplored. With this study, we identify how the degradation of DMF through hydrolysis and thermal decomposition increases the solubility of the perovskite salts. This, in turn, causes a reduction in both the size and concentration of colloids in the precursor solution, resulting in the formation of perovskite films with larger domains and more highly oriented crystals. By identifying the correlation between DMF degradation, solution pH, and perovskite crystallization we illustrate a fundamental, and thus far overlooked mechanism that has tremendous impact on the crystallization of perovskite films. Control of DMF degradation not only governs the reproducibility of perovskite-based optoelectronic devices but also provides a means to greatly reduce their voltage losses, thus overcoming a major challenge on the way to approaching the theoretical efficiency limit for perovskite solar cells.

EXPERIMENTAL PROCEDURES

Full experimental procedures are provided in the Supplemental Information.

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Supplemental Information

Unveiling the Influence of pH on the

Crystallization of Hybrid Perovskites,

Delivering Low Voltage Loss Photovoltaics

Nakita K. Noel, Martina Congiu, Alexandra J. Ramadan, Sarah Fearn, David P. McMeekin, Jay B. Patel, Michael B. Johnston, Bernard Wenger, and Henry J. Snaith
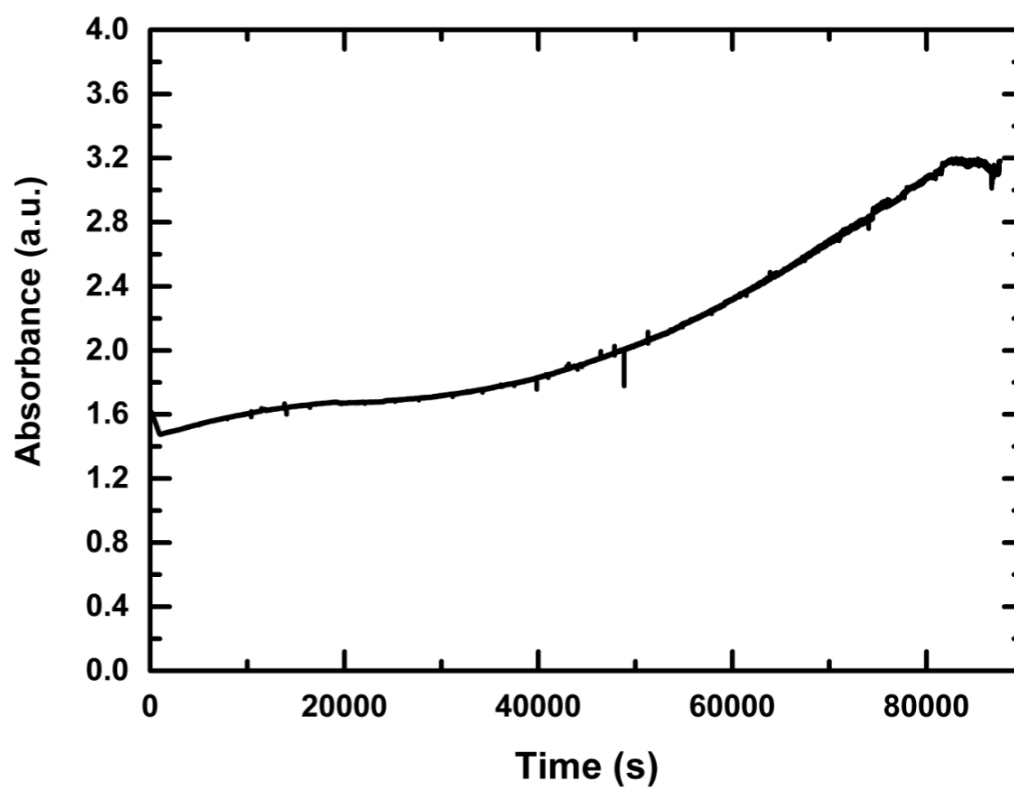
Supplemental Data:

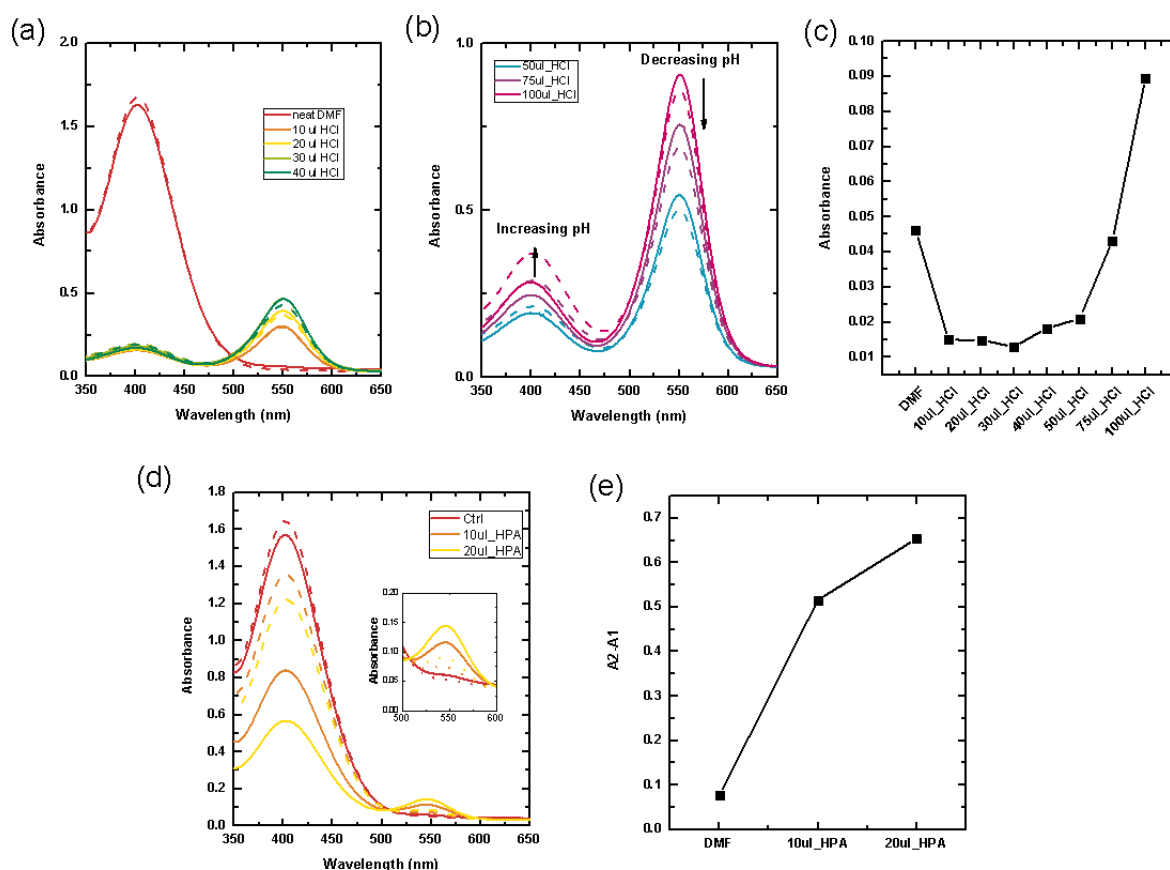**Figure S1:** Absorbance of thymol blue in DMF at 402 nm when solution is held at 95 °C and measured continuously for 24 hrs.

Figure S2: (a), (b) and (d) Absorption spectra of thymol blue in neat, and acidified DMF before (solid lines) and after (dashed lines) temperature cycling. (c) and (e) Difference in the peak 402 nm absorbance before and after temperature cycling.
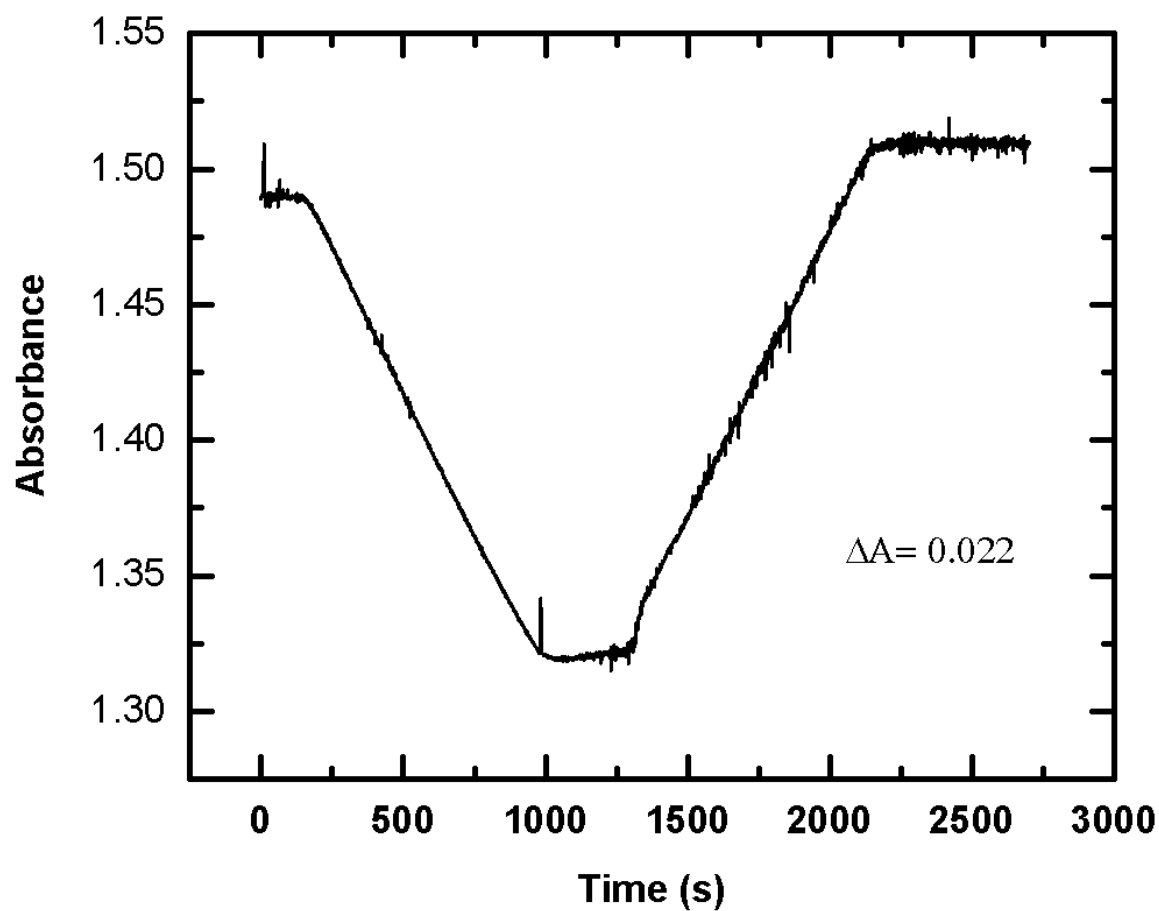
Figure S3: Evolution in the absorbance of thymol blue in DMF + 100 µL/mL of H_2O at 402 nm, with time and temperature.
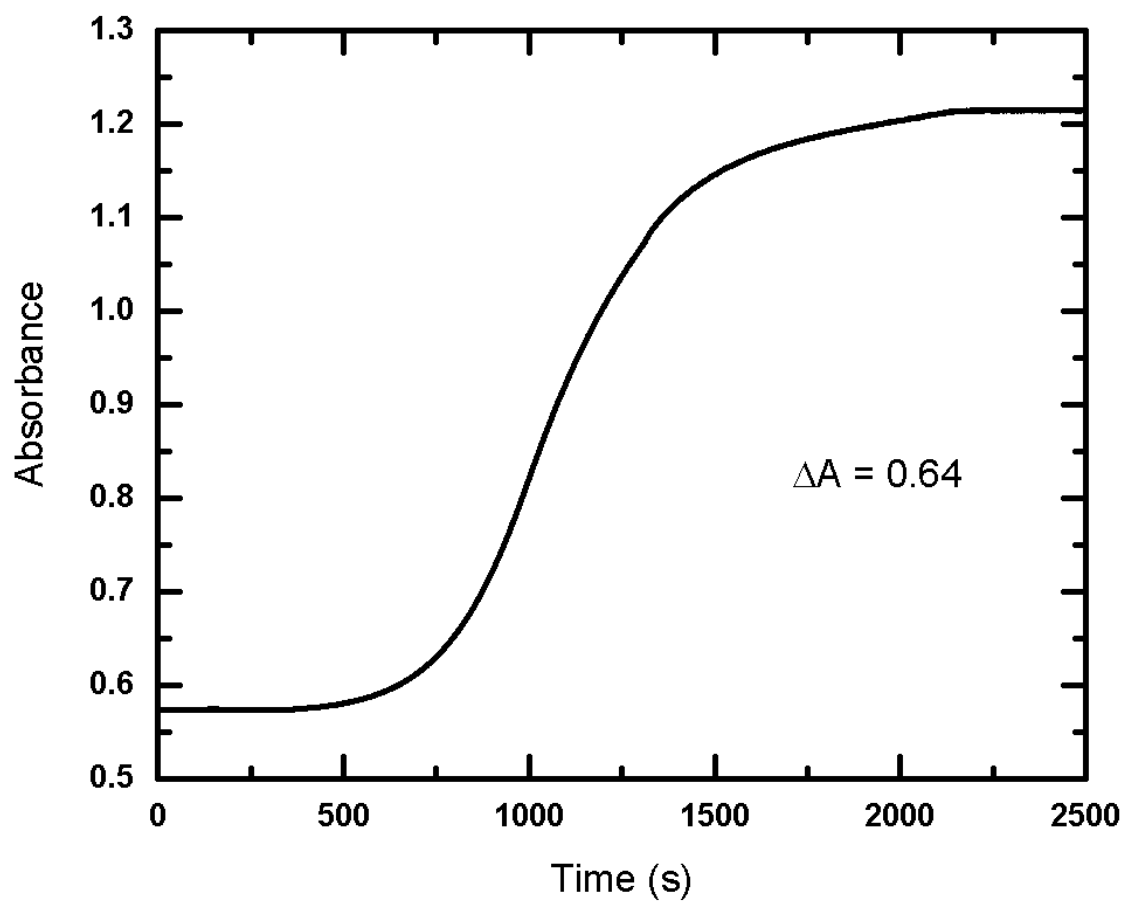
Figure S4: Evolution in the absorbance of thymol blue in DMF with 20 µL of HPA at 402 nm, with time and temperature.
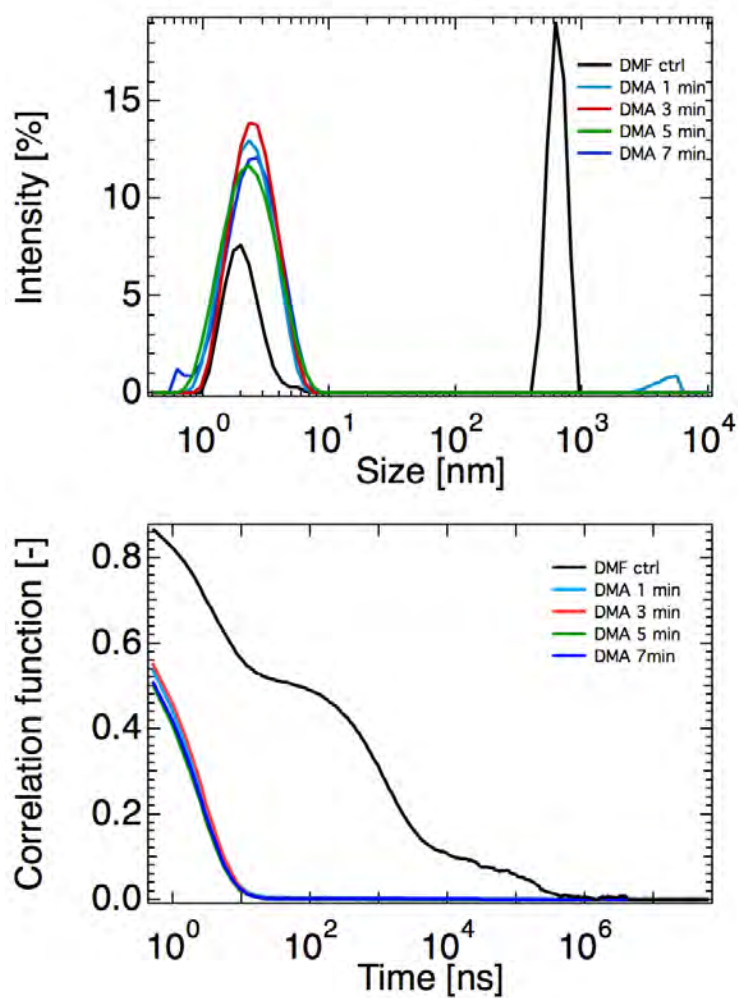
Figure S5: Dynamic Light Scattering. Change in the colloid size and distribution of $CH_3NH_3PbI_{3-x}Cl_x$ with the addition of dimethylamine, bubbled into the solution for 0, 1, 3, 5 and 7 minutes.
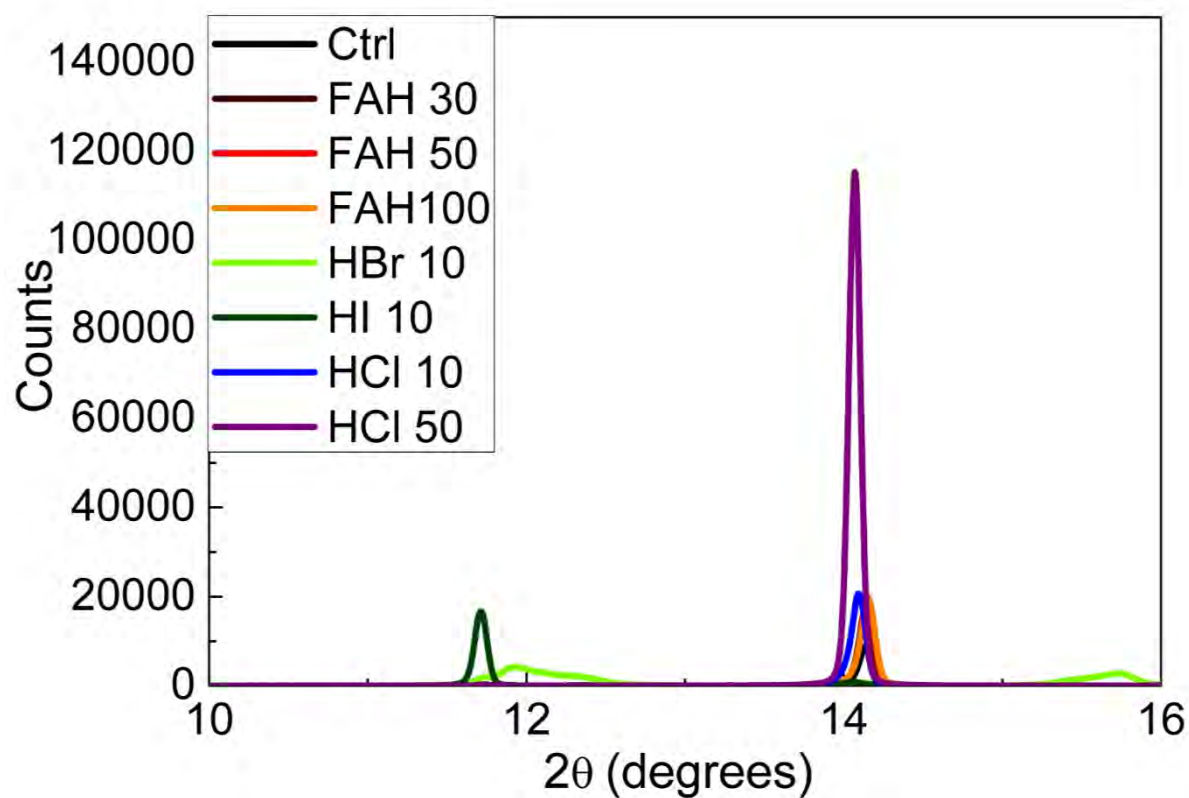
Figure S6: X-Ray Diffraction. X-ray diffraction patterns of $CH_3NH_3PbI_3$ films fabricated from neat DMF (ctrl), and DMF acidified with varying amounts of formic acid (FAH) and hydrohalic acids.
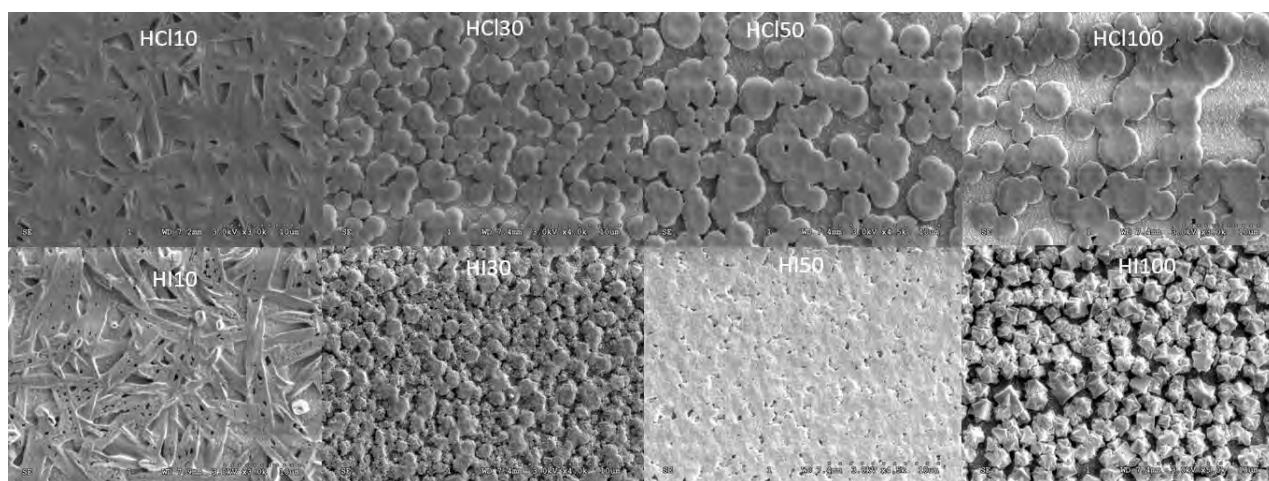
Figure S7: Scanning Electron Microscopy. SEM images of $CH_3NH_3PbI_{3-x}Cl_x$ films fabricated with various amounts of HI and HCl added to the precursor solution.
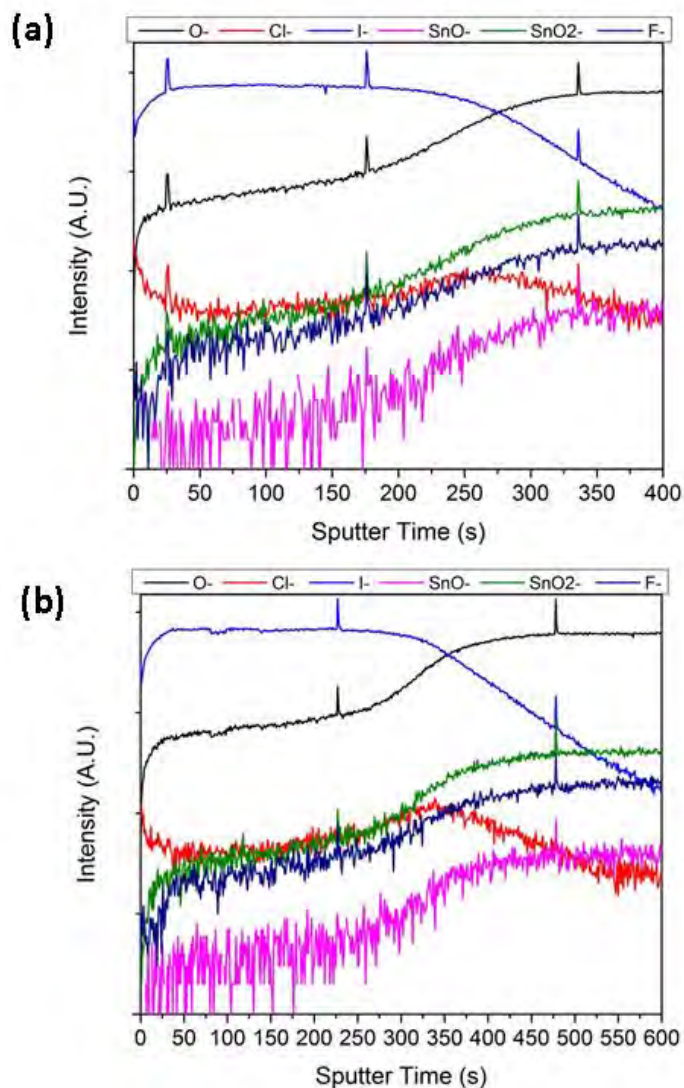
Figure S8: Secondary Ion Mass Spectroscopy. Spectrographs of films of $CH_3NH_3PbI_{3-x}Cl_x$ films fabricated from precursor solutions with (a) neat DMF, and (b) DMF and 30 μL/mL of formic acid.
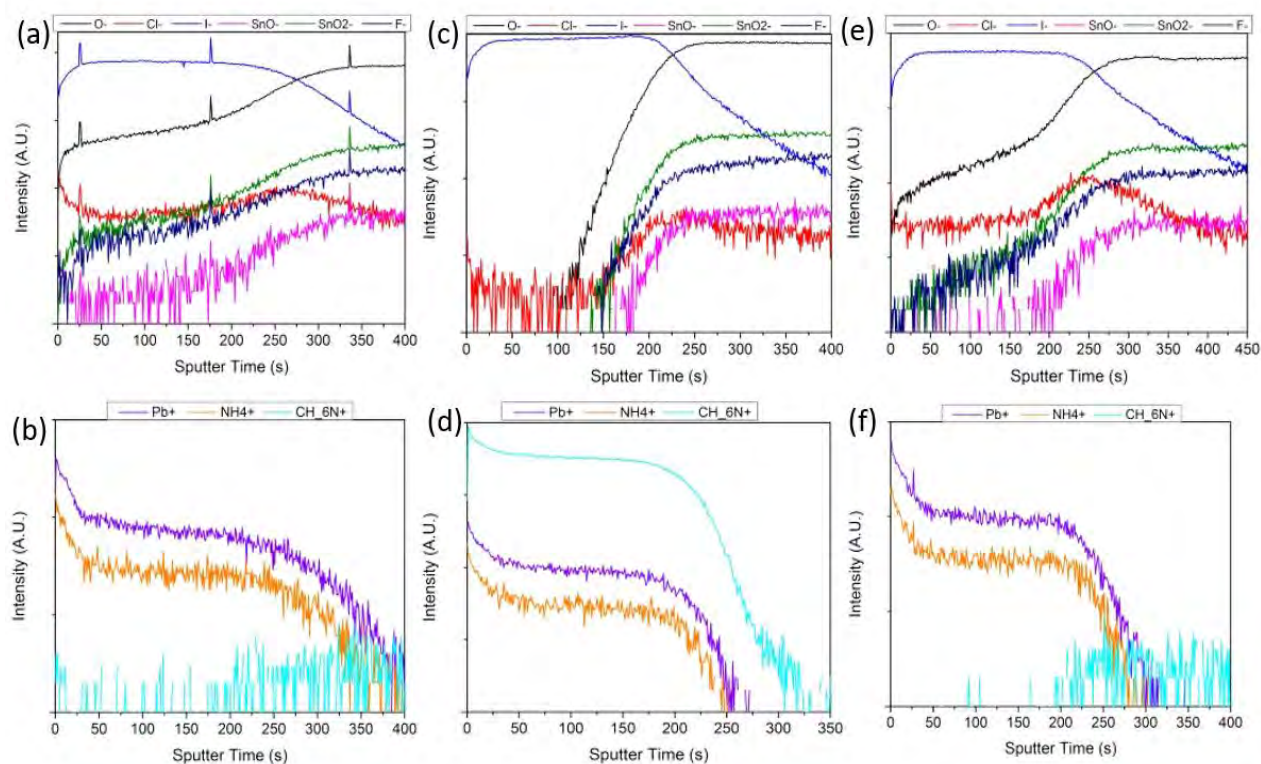
Figure S9: Secondary Ion Mass Spectroscopy. Spectrographs of films of $CH_3NH_3PbI_{3-x}Cl_x$ films fabricated from precursor solutions with neat DMF (a)and (b), and 0.02 vol.% of HCl (c) and (d), and HI (e) and (f).
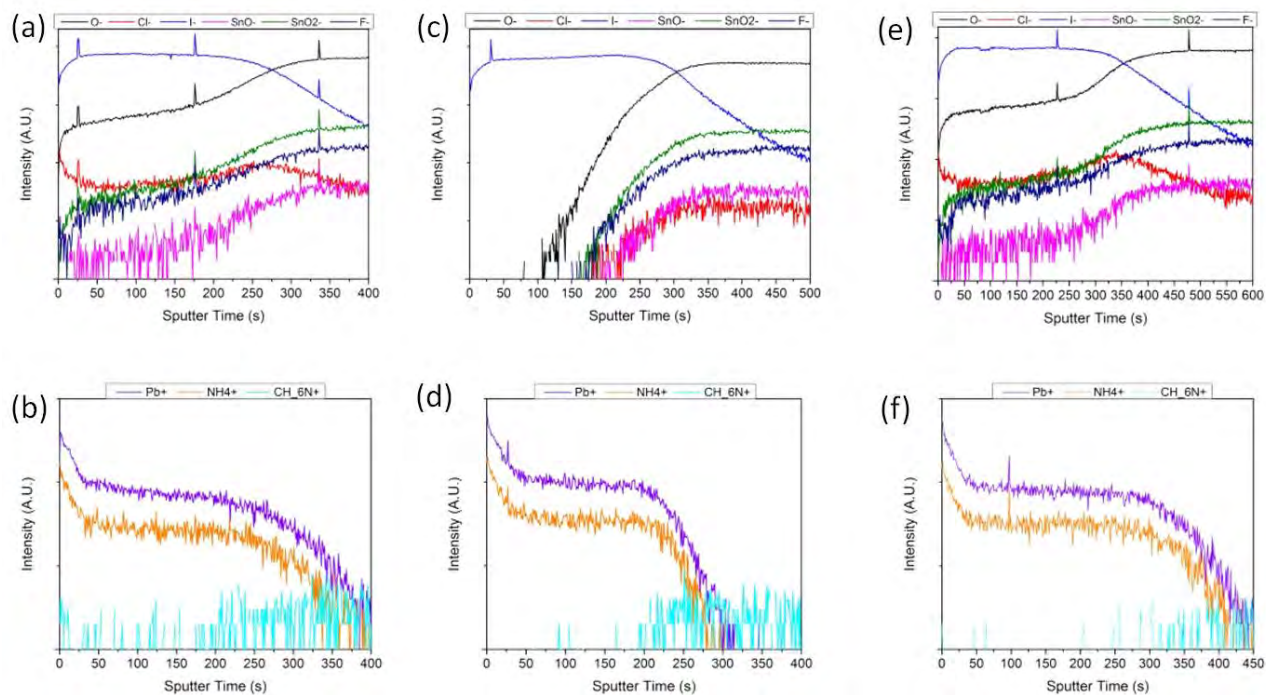
Figure S10: Secondary Ion Mass Spectroscopy. Spectrographs of films of $CH_3NH_3PbI_{3-x}Cl_x$ films fabricated from precursor solutions with neat DMF (a)and (b), and 0.02 vol.% of HPA (c) and (d), and formic acid (e) and (f).
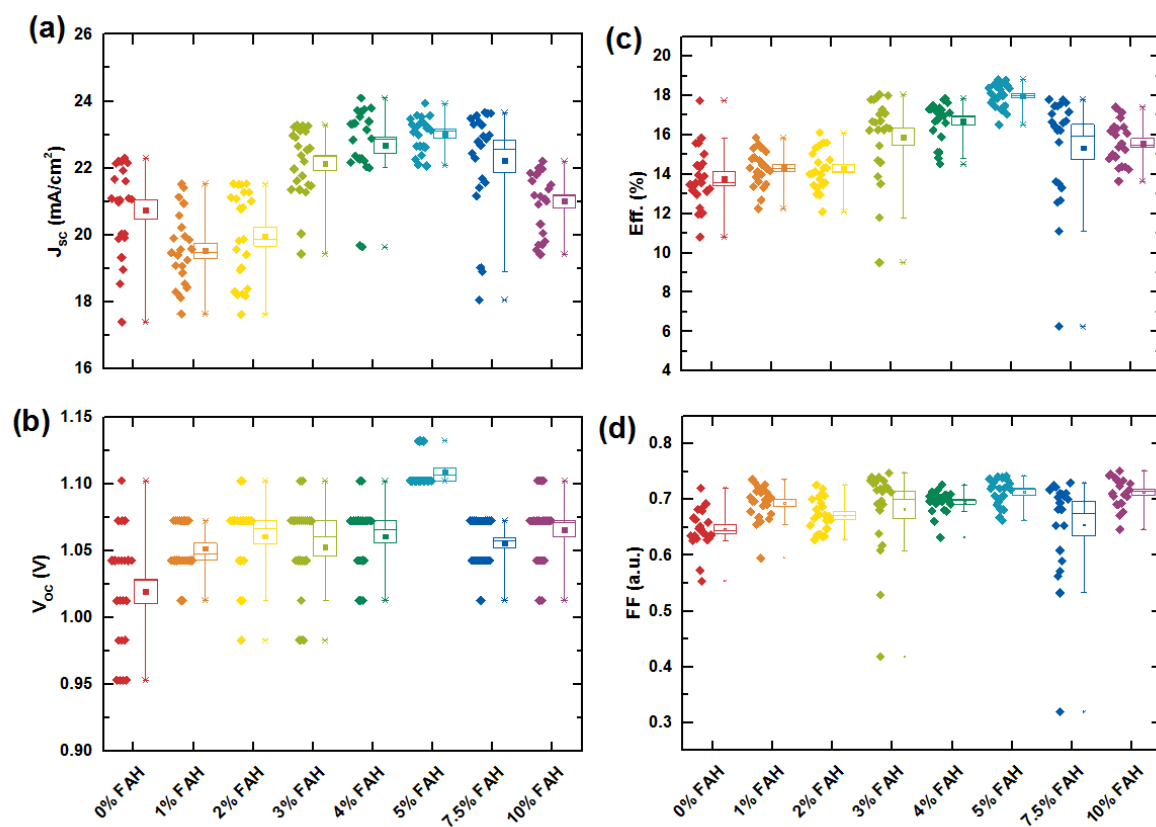
Figure S11: Statistics on the performance parameters of solar cells fabricated from neat and acidified DMF-based $CH_3NH_3PbI_{3-x}Cl_x$ precursor solutions.
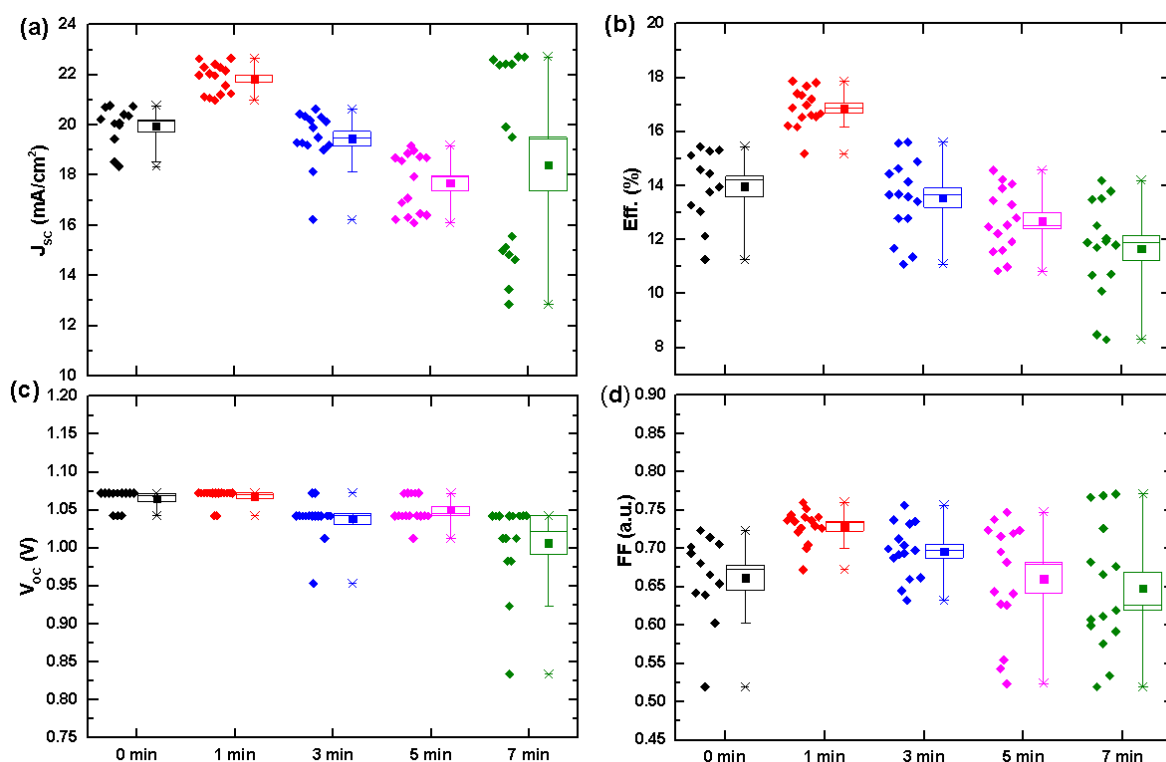
Figure S12: Statistics on the performance parameters of solar cells fabricated from neat and alkalinized DMF-based $CH_3NH_3PbI_{3-x}Cl_x$ precursor solutions. Solutions were bubbled with dimethylamine for 1 min, 3 min, 5 min and 7 min.
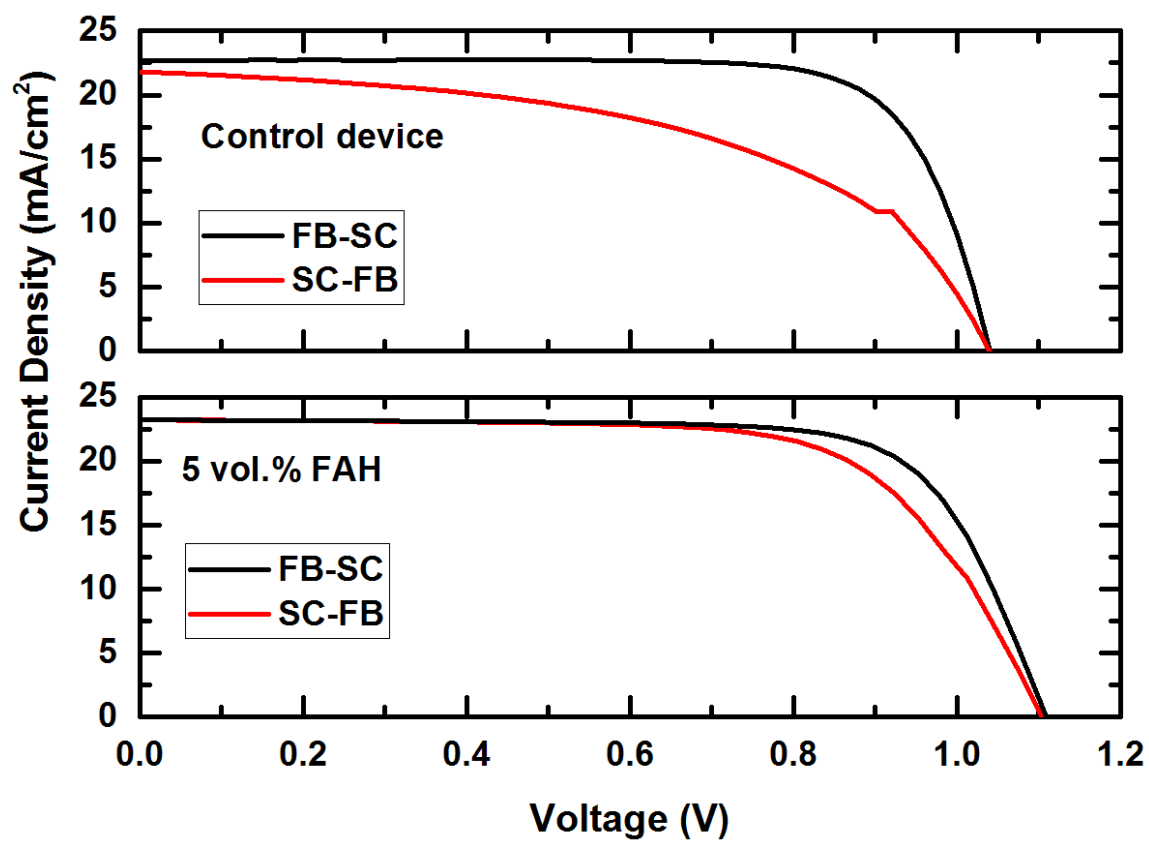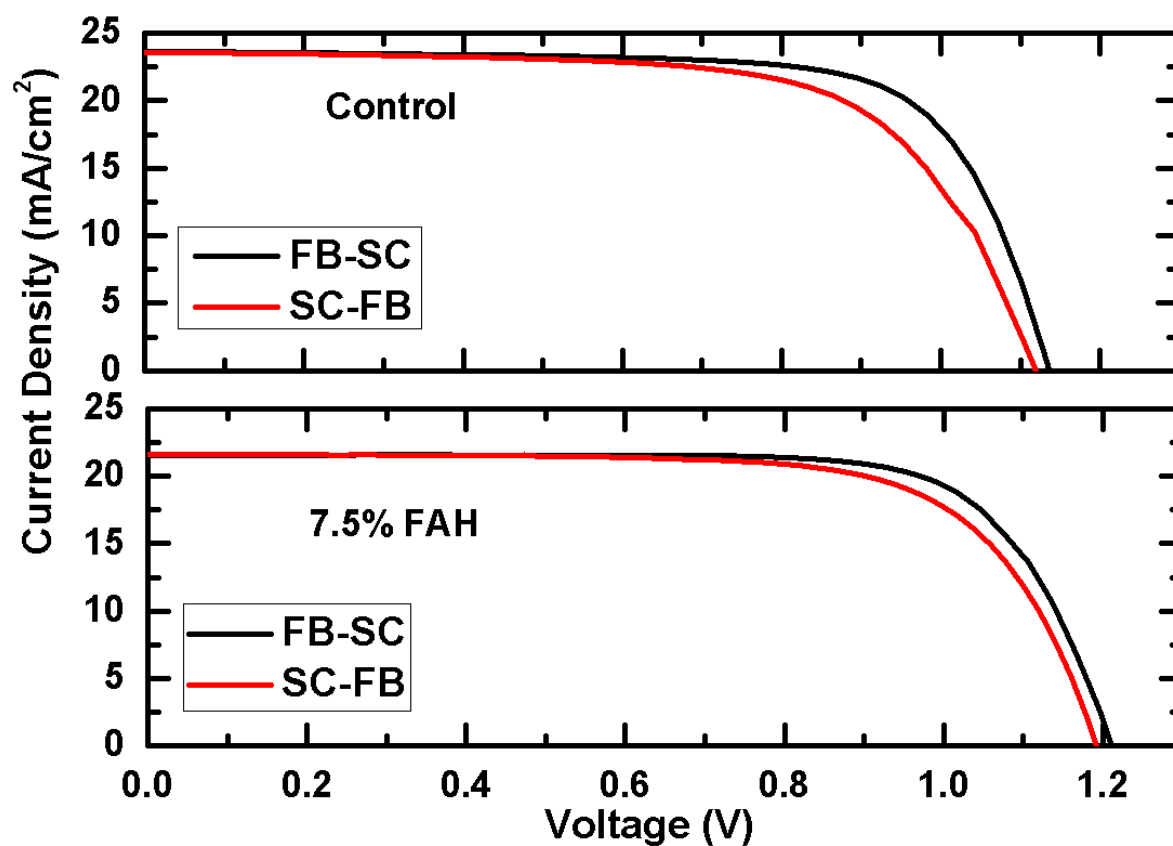
Figure S13: Current-Voltage curves of the solar cells fabricated from a neat, and an acidified precursor solution of $CH_3NH_3PbI_{3-x}Cl_x$.
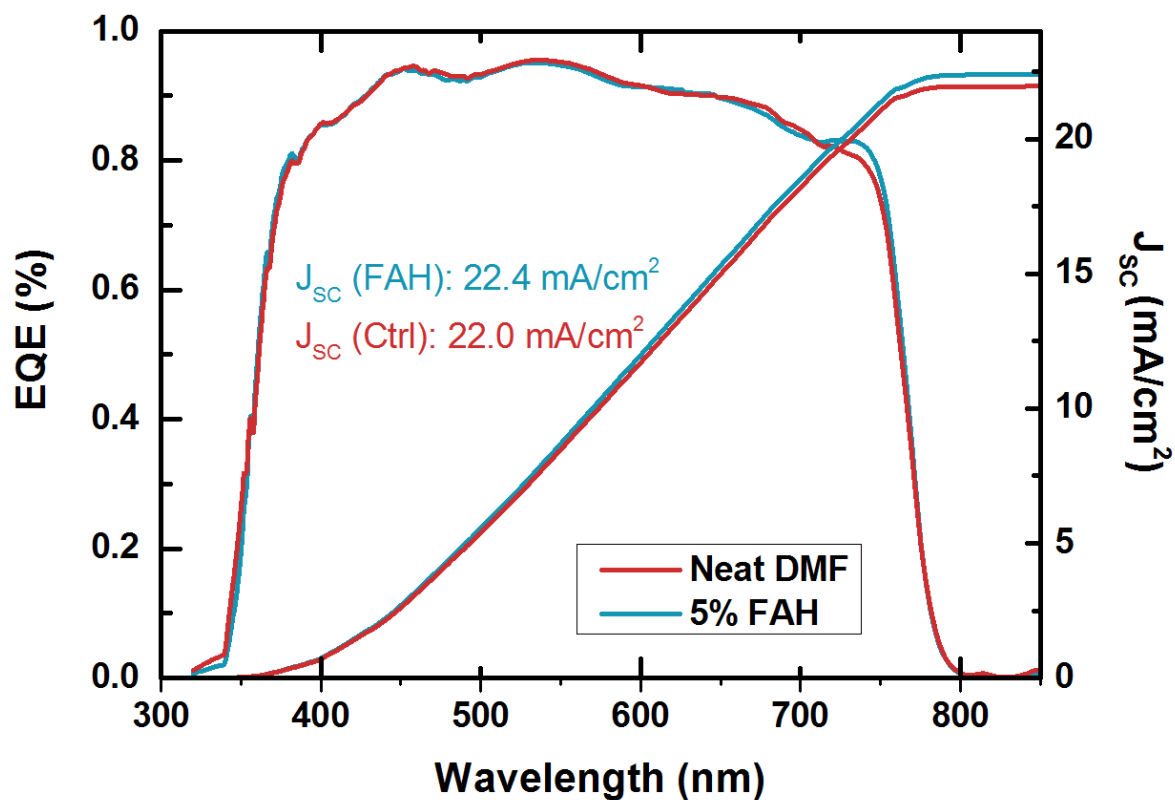
Figure S14: External Quantum Efficiency of champion $CH_3NH_3PbI_{3-x}Cl_x$ devices fabricated from neat

DMF and from DMF with formic acid, giving integrated current densities of 22.0 mA/cm^2 and 22.4

mA/cm^2 respectively. Both values are in good agreement with the J_{SC} obtained during the current-

voltage characterisations.
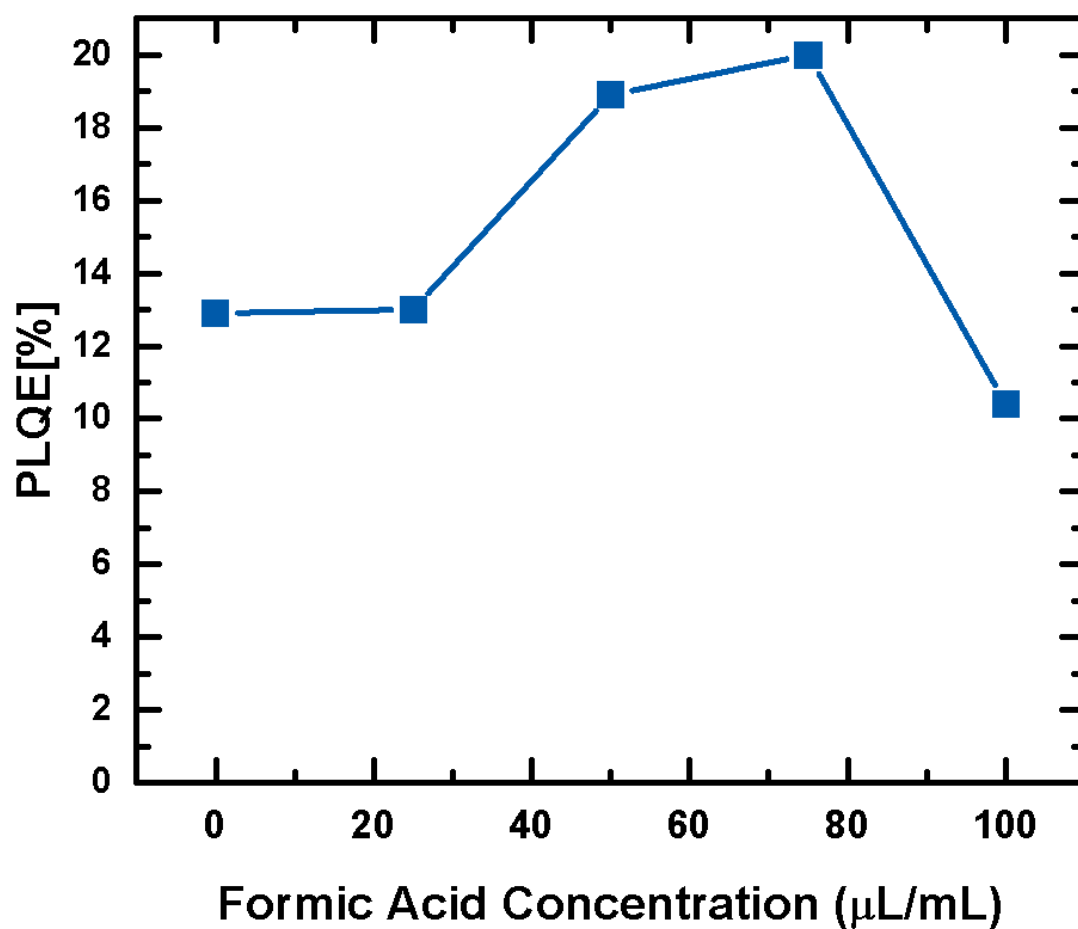
Figure S15: Photoluminescence quantum efficiencies of $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ films fabricated from precursor solutions with varying concentrations of formic acid.
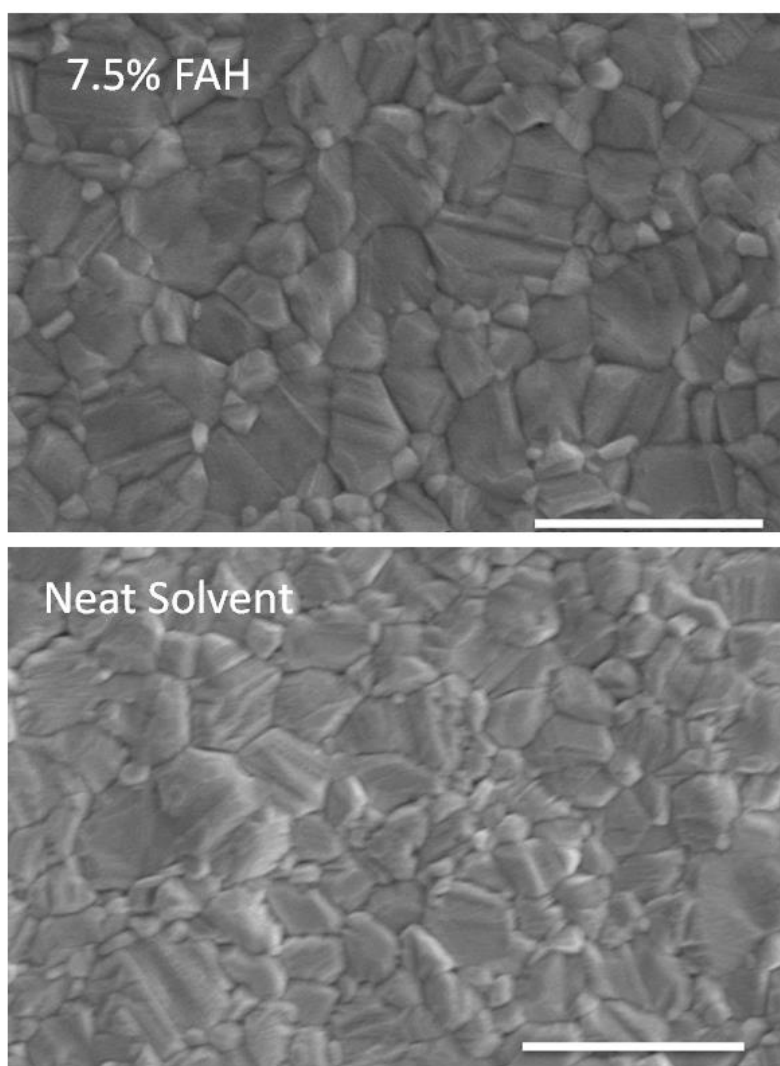
Figure S16: SEM images of $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ films fabricated from precursor solutions with

and without formic acid.
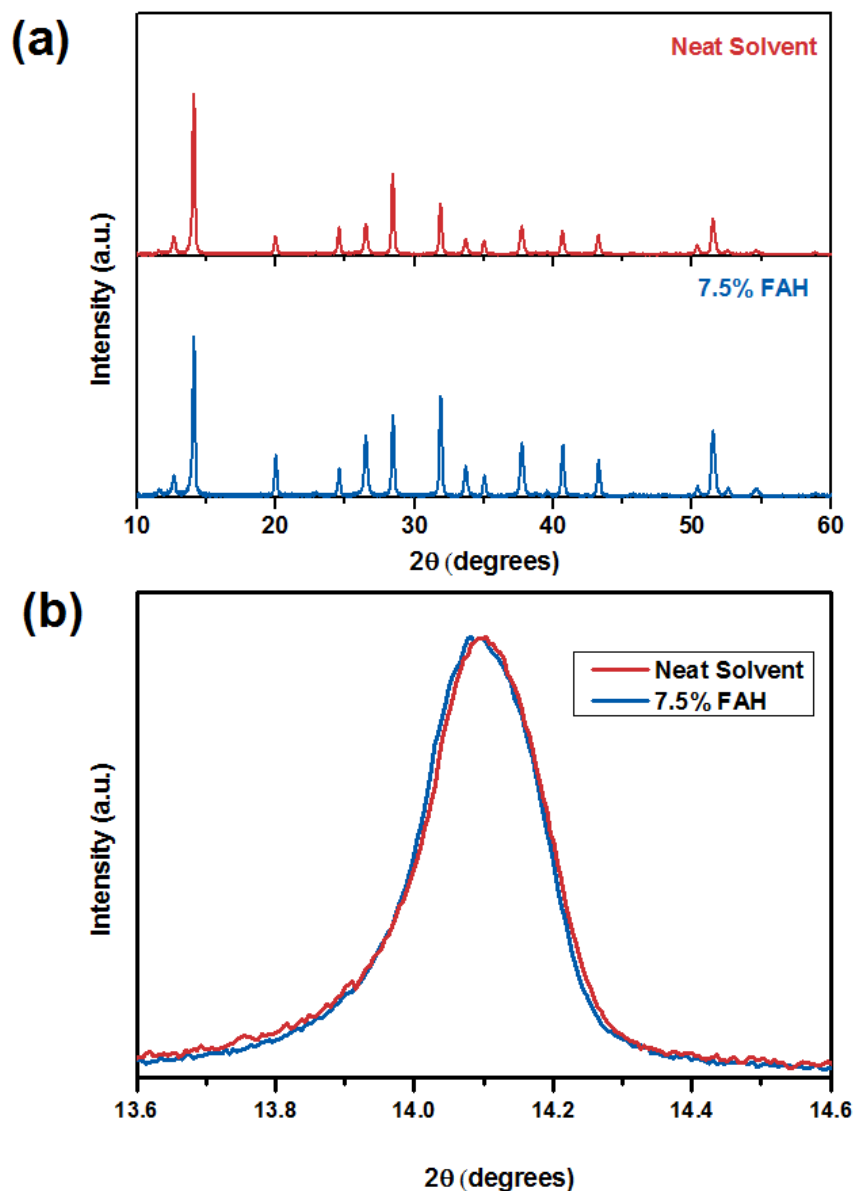
Figure S17: (a) X-Ray diffraction patterns of films of $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ films fabricated from precursor solutions made with neat solvent, and solvent with 7.5 vol.% formic acid. (b) Close-up view of the 110 peak of the perovskite films fabricated with and without acid, showing almost identical peak widths and intensities. The difference in the ratio of the intensities of the peaks shown in the films fabricated with and without formic acid, indicates a slight difference in the orientation of the crystals in the film.

Figure S18: Forward and reverse current-Voltage curves of the solar cells fabricated from a neat, and

an acidified precursor solution of the mixed-cation, mixed-halide perovskite $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$.
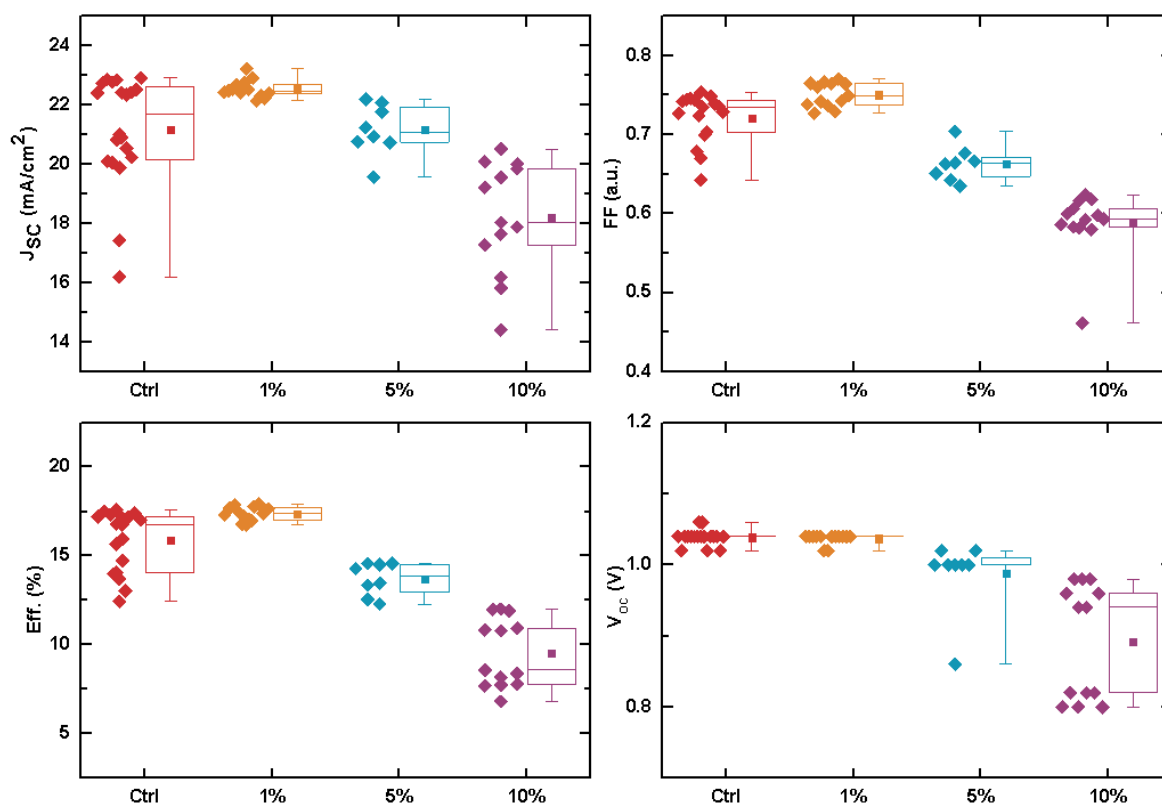
Figure S19: Performance parameters for a batch of $CH_3NH_3PbI_{3-x}Cl_x$ devices where the compact layer was pre-treated with acidified DMF at different concentrations of FAH.
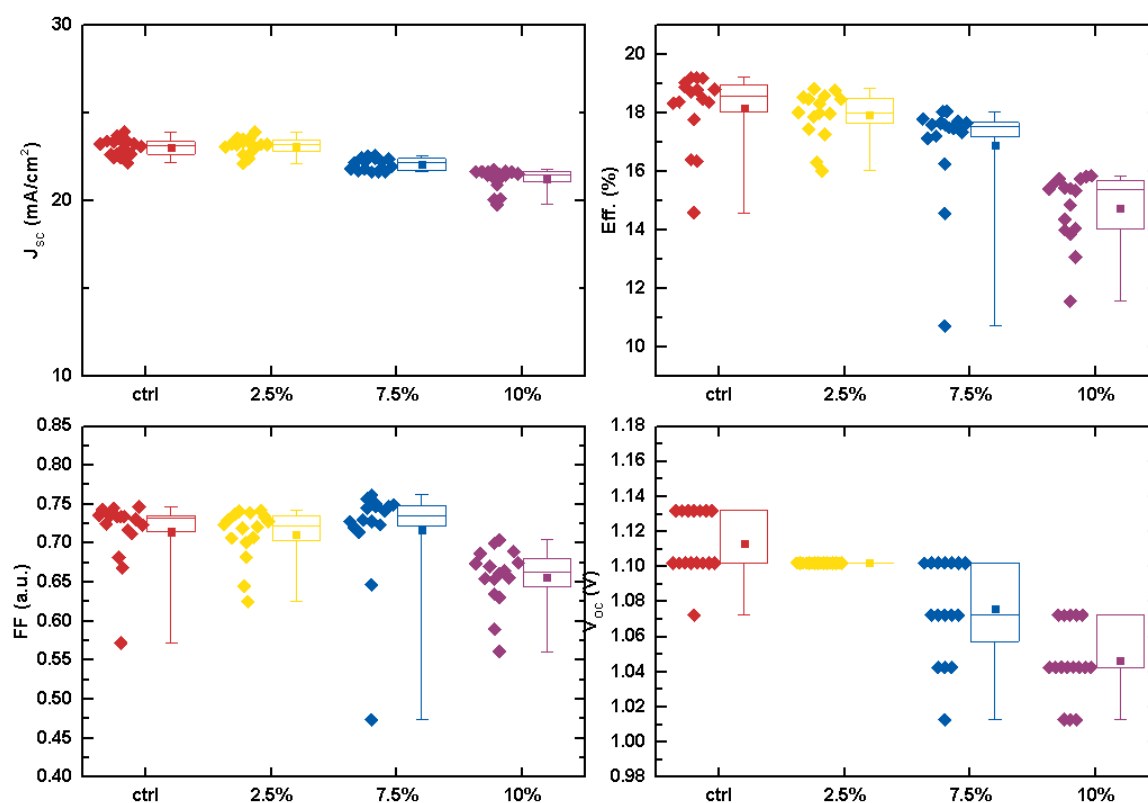
Figure S20: Performance parameters for a batch of $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ devices where the compact layer was pre-treated with acidified DMF at different concentrations of FAH.
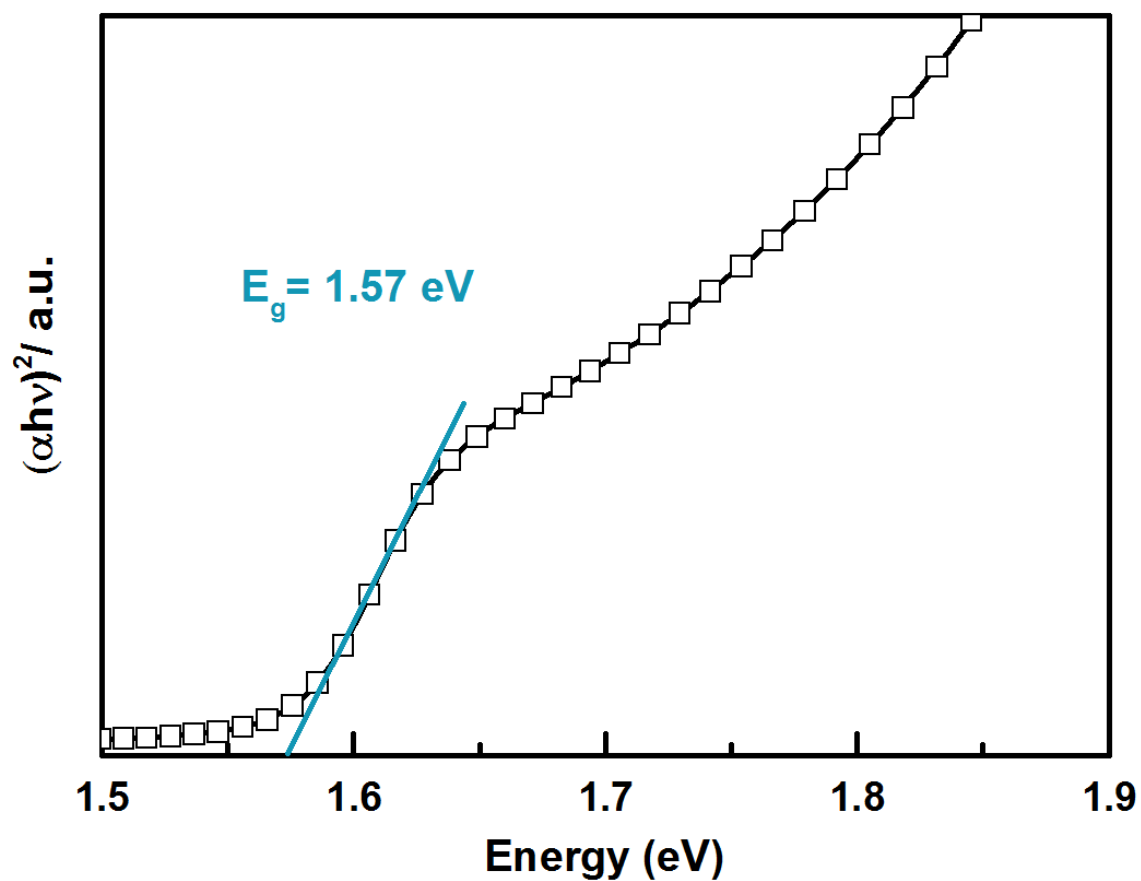
Figure S21: Tauc plot showing the band gap determination of the mixed-cation, mixed-halide perovskite $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$, assuming a direct band gap material.
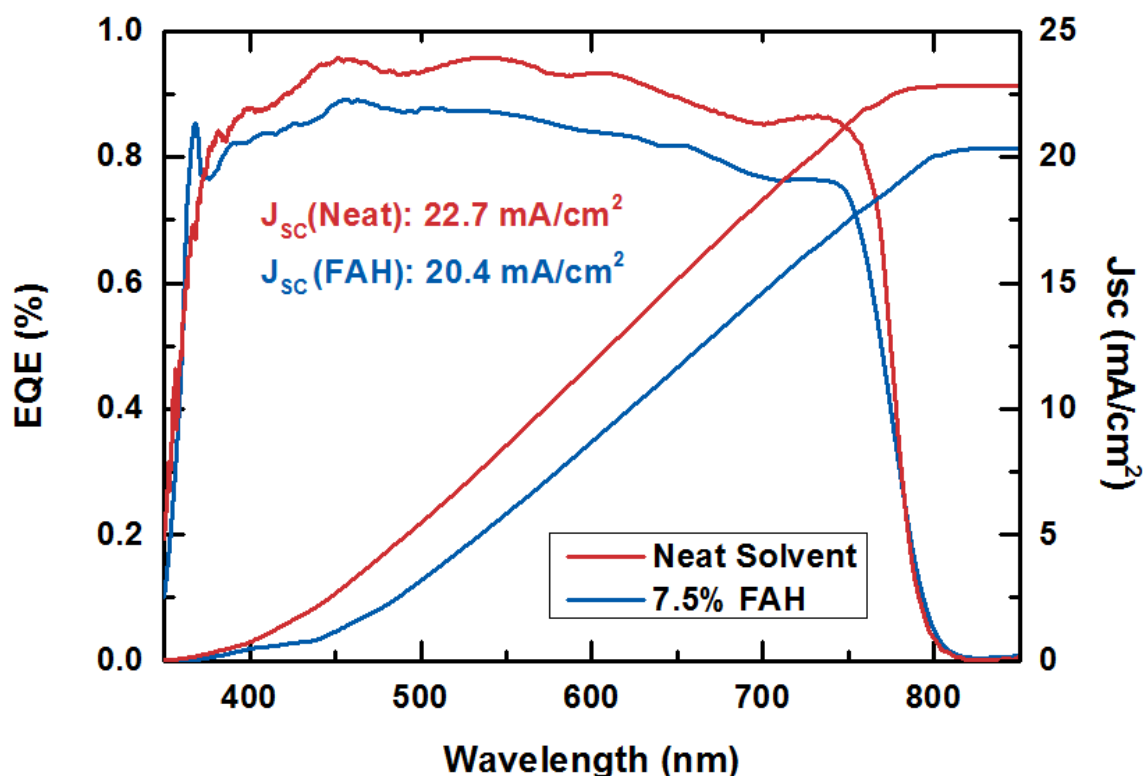
Figure S22: External Quantum Efficiency of champion $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ device fabricated with neat and acidified solvents, giving integrated current densities of 20.4 mA/cm^2 and 22.7 mA/cm^2 respectively.

Supplemental Experimental Procedures:

Synthesis of Methylammonium Iodide: Methylammonium iodide (MAI) was synthesised according to previously published methods.(Lee et al., 2012) Briefly, methylamine (33 wt.% in ethanol, Sigma Aldrich) was added to HI (57 wt.% in H_2O, Sigma Aldrich) in ethanol with stirring, under inert conditions. The mixture was left stirring for 4 hours at room temperature. The MAI was crystallised by driving off the ethanol using a rotary evaporator. The MAI was recrystallized from warm ethanol and washed using diethyl ether, followed by vacuum drying. After drying the MAI was kept in a desiccator until use.

Dimethylamine Bubbling: A 38 wt.% precursor solution of $CH_3NH_3PbI_{3-x}Cl_x$ was prepared by dissolving MAI and $PbCl_2$ in DMF at a 3:1 molar ratio. After the powders were dissolved, dimethylamine was bubbled into the solution using previously reported methodology. (Noel et al., 2017) Bubbling was done for for 1, 3, 5 or 7 minutes as required.

Fabrication of Planar Heterojunction Solar Cells: Planar heterojunction solar cells were fabricated utilising previously published methods.(Eperon et al., 2013; Noel et al., 2014) Concisely, FTO-coated glass sheets ($7\Omega cm^{-1}$ Hartford Glass) were etched with zinc powder and HCl (3M) to obtain the required electrode pattern. The sheets were then washed with soap (2% Hellmanex in water), deionized water, acetone, ethanol and isopropanol, and finally treated under oxygen plasma for 10 min to remove the last traces of organic residues. A compact layer of SnO_2 was then deposited on the glass using a modified version of the methodology presented by Anaraki et al.(Anaraki et al., 2016) Briefly, $SnCl_4.5H_2O$ was dissolved in isopropanol at a concentration of 0.05M and stirred at room temperature for 1 hour. The solution was then spincoated onto the substrates at 3000 rpm, after which the substrates were annealed at 180˚C for 1 hour before being left to naturally cool down to room temperature. The substrates were then immersed into a chemical bath , which consisted of $SnCl_2 \cdot 2H_2O$ (Sigma-Aldrich) in deionised water (0.012 M), 20.7 mM urea (Sigma-Aldrich), 0.15 M HCl (Fisher scientific) and 2.87 µM 3-mercaptopropionic acid (Sigma-Aldrich). The substrates were kept in an oven at 70 °C for 180 min, after which they were sonicated in deionised water for 2 minutes. They were then washed with ethanol and annealed at 180 °C for 60 min. The required amount of formic acid or dimethylamine was added to the 38 wt.% perovskite precursor solution in DMF such that the overall concentration of the solution was kept constant (3:1 molar ratio of MAI: $PbCl_2$) ($PbCl_2$, Sigma Aldrich 98 %). The precursor solution was then spin coated directly onto the substrate at 2000 rpm in a nitrogen filled glove box. The substrate was then left to dry under N_2 for 10 min before being placed on a hotplate at 90°C where it was annealed for 180 min. For the mixed-cation, mixed halide perovskite, a 1.25M solution of $FA_{0.83}MA_{0.17}Pb(I_{0.83}Br_{0.17})_3$ was prepared using a 4:1 vol.:vol. ratio of DMF:DMSO. The solution was spincoated onto the desired substrate at 1000 rpm for 10 s, followed by 6000 rpm for 35 s. 100 µL of anisole was dropped onto the substrate 35 s after the beginning of the spincoating. The films were then annealed at 100 °C for 60 min. After annealing, the substrate

was allowed to cool down to room. Subsequently, the hole transport material (HTM) was dissolved in chlorobenzene (85 mg/mL with 30 mol % Li-TFSI and 80 mol % tBP as additives), and was spin coated onto the perovskite substrates at 2000 rpm. 100 nm thick silver electrodes were then deposited under high vacuum (10^{-6}) through a shadow mask.

UV-Vis Spectrophotometry: The steady-state absorption spectra were acquired with a Perkin-Elmer Lambda 1050 UV/Vis/NIR spectrophotometer. A stock solution of thymol blue was prepared by dissolving 1 mg/mL of thymol blue (Sigma Aldrich) in dimethyl sulfoxide (DMSO). The thymol blue solution was then added to neat and acidified anhydrous dimethylformamide (DMF) such that the concentration of indicator in the final solution was 10 vol.%. Measurements were taken in a temperature controlled cuvette holder with constant stirring. All steady state absorption spectra were acquired at 25 ˚C. For temperature dependent measurements, the solution was held at 25 ˚C for 2 minutes, after which the solution was gradually heated (over 15 minutes) to 95 ˚C, where it was held for 5 minutes, before being cooled back down to room temperature.

Transmission and absorption spectra of thin films were collected using a Varian Cary 300 UV-Vis spectrophotometer with an integrating sphere.

Dynamic Light Scattering (DLS): The DLS experiments were performed with a Zetasizer Nano ZS instrument (Malvern Instruments, UK), equipped with a 633 nm He-Ne laser. In a typical experiment each sample is measured three times with 15 runs per measurement (10-15 seconds per run). The temperature was stabilised during 120 seconds prior to the acquisition of the data. All samples were measured in the backscattering mode. Most samples showed high polydispersity, therefore the autocorrelation decays are fitted with a multimodal distribution model.

Secondary Ion Mass Spectroscopy:

Secondary ion mass spectrometry (SIMS) analyses were performed on an IONTOF ToF-SIMS V instrument. The primary ion beam was a 25keV Bi^+ in BAM (bunched to 4 pulses to avoid detector

saturation). The sputter beam was Cs+ at 1keV and a beam current of 75nA. The analytical area was 100um^2 with a sputter crater of 300um^2.

Current-Voltage Characterisation: Solar cell performance was measured using a class AAB ABET sun 2000 solar simulator that was calibrated to give simulated AM 1.5 sunlight at an irradiance of 100 mW/cm^2 . The irradiance was calibrated using an NRELcalibrated KG5-filtered silicon reference cell. Currentvoltage curves were recorded using a sourcemeter (Keithley 2400, USA). All solar cells were masked with a metal aperture that was used to define the active area of the devices, which in this case was 0.0925 cm^2. All devices were stored in a desiccator in the dark for 12 h prior to testing.

External Quantum Efficiency Measurements: External quantum efficiency (EQE) was measured via custom build Fourier transform photocurrent spectrometer based on a Bruker Vertex 80v Fourier Transform Interferometer. Devices were illuminated with an AM1.5 filtered solar simulator. Devices were calibrated to a Newport-calibrated reference silicon solar cell with known external quantum efficiency. The devices were masked with a metal aperture with a defined active area, 0.0919 cm^2.

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Exhibit 36

RESEARCH ARTICLE

Regeneration of aged DMF for use in solid-phase peptide synthesis

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Dimethylformamide (DMF), which is still the most commonly used solvent for Fmoc-SPPS, has the potential for degradation over time on exposure to air (and water vapour) and storage, to give dimethylamine and formic acid impurities. In particular, dimethylamine can lead to unwanted deprotection of the fluorenylmethyloxycarbonyl (Fmoc) group during, for example, the initial loading of Fmoc amino acids in SPPS, which leads reduced calculated loading values. We have found that treatment of such aged DMF by simple sparging with an inert gas (N_2), or vacuum sonication, can regenerate the DMF in order to restore loading levels back to those found for newer, fresh, DMF samples.

KEYWORDS
DMF regeneration, Fmoc group, resin loading, SPPS

1 | INTRODUCTION

Dimethylformamide (DMF) is one of the most commonly used solvents in solid-phase peptide synthesis (SPPS). In recent years, there has been increasing concern over the environmental, health, and safety (EHS) profile of DMF, with many solvent selection guides categorising DMF as hazardous.[1-3] Furthermore, DMF is also suspected to possess teratogenic properties, and in recent years, has become subjected to regulations such as the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation along with N,N-dimethylacetamide (DMAc), and N-methylpyrrolidinone (NMP).[4,5] In light of the aforementioned issues, there has been an increase of interest within the peptide science community in the use of greener solvents for SPPS. Albericio, North, and Lopez recently reported successful synthesis of peptides using green

SPPS strategies and proposed several different possible greener alternatives to DMF for solid-phase synthesis.[6-15] Despite these issues, DMF remains a popular solvent for fluorenylmethyloxycarbonyl-based SPPS (Fmoc-SPPS)[6,16] and is likely to remain a popular solvent in academic settings for the foreseeable future.

It has long been known that DMF is not stable over a long period of time and, in the presence of water, degrades to the secondary amine, dimethylamine (DMA) and formic acid (FA) (Figure 1), though storage under an inert gas has been suggested as a possible way of reducing degradation.[17,18] During synthesis, use of newly manufactured DMF with minimal DMA content to reduce unwanted reactions and storing DMF under an inert atmosphere after every use to mitigate DMA formation are also recommended.[18] In situations where the regular purchase of newly manufactured DMF is not possible, it may be necessary to treat aged DMF instead to remove any DMA.[19]

Because of unwanted fluorenylmethyloxycarbonyl (Fmoc) removal, the presence of significant amounts of DMA in DMF can be particularly problematic for SPPS that utilises the base-labile Fmoc-protecting group for the temporary protection of the α-amino group.[19,21-24] Some protocols recommend treating DMF to reduce DMA content prior to synthesis, which include sparging DMF with an inert gas such as nitrogen.[19]

A rapid colourimetric test involving the use of Sanger's reagent (dinitrofluorobenzene [DNFB]) for the detection of DMA in DMF has also been described in the literature. During the DNFB test, a DMF
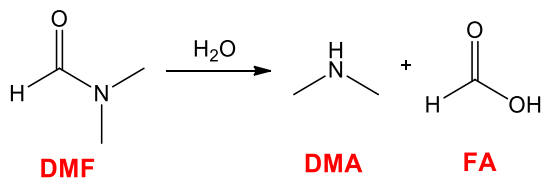

FIGURE 1 Scheme for the hydrolysis of dimethylformamide (DMF) to dimethylamine (DMA) and formic acid (FA)[18,20]

TABLE 1 Range of solvents used in these studies (other than dimethylformamide [DMF])

	Grade and percentage purity
Dichloromethane (DCM)	Analytical grade, $\geq 99.99\%$
Methanol (MeOH)	$\geq 99.5\%$
Ethanol (EtOH)	$\geq 99.8\%$
γ-Valerolactone (GVL)	Food chemicals codex, food grade, $\geq 99.0\%$
N-Formylmorpholine (NFM)	$\geq 99\%$
Cyrene (CYR)	$\geq 99\%$

sample suspected of having a significant amount of DMA is mixed with a solution of 1 mg/mL DNFB in 95% EtOH, in a 1:1 ratio and left to stand in the dark for 30 minute. The absorbance of the mixture is measured versus a DNFB/95% EtOH solution blank. DMF samples that give absorbance values of less than 0.15 are deemed to be of satisfactory purity.[18,25,26]

The initial resin loading, which is typically reported in mmol per gram of resin (mmol.g^{-1}), has been identified as a critical parameter in SPPS.[27] In this technique, the initial anchored unit critically contributes to the final yield of the peptide since it serves as the limiting reagent for all subsequent couplings.[27-29] Following initial loading and during peptide assembly, constant loading or a slight progressive decrease in loading has been described as an indication of good progress of synthesis.[30] After cleavage of the peptide from the solid support, synthetic yields are typically reported based on the loading of the initial anchored unit.[27,28,31-34] Resin loading can be estimated spectrophotometrically with ultraviolet–visible (UV–Vis) spectroscopy-using methods based on DBU/DMF and dibenzofulvene (DBF) generation or piperidine/DMF and DBF-piperidine adduct ($\lambda_{max} \approx 267$ nm, 290 nm, 301 nm) generation.[35-37]

Overloading of resins is known to potentially cause problems during synthesis.[27] Lower loading levels are more commonly employed in SPPS since high loadings have been associated with synthetic failures.[28] In general, high loadings introduce greater steric crowding and as a result, decreased reactive site accessibility within the resin. Overloading has also been attributed to increased likelihood of intermolecular interactions between the growing peptide chains and as a consequence, aggregation and failure of synthesis.[27,28,38]

DMA has been identified to be able to potentially remove Fmoc groups from Fmoc-protected amines.[17,19,39] If aged DMF is used during Fmoc-SPPS, its presence can lead to unwanted Fmoc removal, particularly during the initial loading and peptide assembly stages of SPPS.[18] Another consequence of unwanted Fmoc removal during synthesis is a possible decrease in purity of the final peptide product.[19,39]

2 | MATERIALS AND METHODS

The initial loading was carried out using Fmoc-Gly-OH ($\geq 99.9\%$) from Iris Biotech GmbH in disposable 2 mL MultiSynTech GmbH peptide reactors (Part# V020PE061) and Luer stoppers (Part# V000LS100). 2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU; $\geq 99.0\%$) and Rink Amide-MBHA-PS (RA-MBHA resins, 0.580 mmol.g^{-1} capacity, crosslinking 1% DVB) resins were donated by IPSEN Manufacturing Ireland Ltd. Piperidine ($\geq 99.5\%$ AcroSeal®, Lot# A0362067) and DBU ($\geq 99.0\%$, Lot# BCBL8308V) were purchased from Acros Organics and Sigma Aldrich Ireland, respectively. N,N-diisopropylethylamine (DIPEA; $\geq 99\%$, Lot# STBF0608V) was also purchased from Sigma Aldrich Ireland. UV–Vis spectroscopy studies were carried out using 1 mL volume and 1 cm path length Hellma Analytics Quartz SUPRASIL cuvettes on a Hitachi U-2900 spectrophotometer and the software used was the UV Solutions software. Vacuum sonication was carried out with a Decon F5100b sonicator and a BUCHI Labortechnik AG V-700 pump. The silica gel (40–63 µm, pH 6.7, Lot# 17D074111) and the basic alumina (50–200 µM, pH 9–10, Lot# A0386697) were purchased from VWR and Acros Organics, respectively. The DNFB ($\geq 98\%$, Lot# A0392098) for the colourimetric DNFB tests was purchased from Acros Organics. FA (>98%, Lot# BCBP4740V) was purchased from Fisher Scientific. Measurements of refractive indices (n^{20}_D) were carried out using an Index Instruments Automatic Refractometer model PTR2a at 20°C, using a sodium yellow light source (589 nm) and set at continuous mode. For statistical analyses and data processing, PRISM 6.0 was used to analyse the results using analysis of variance (ANOVA) with Tukey–Kramer's test ($\alpha = 0.05$) and to detect possible data outliers (Q = 1% or $\alpha = 0.05$).[40,41] Values of p that are less than 0.05 mean that there is a statistically significant difference, which is also marked by asterisks. The solvents were used before the stated date of expiration and stored in dark safety storage cabinets for flammable organic solvents away from direct sunlight.

The age and therefore the date of manufacture of the different DMF samples was central to the study and are mentioned below (Table 2). Manufacture dates were obtained by direct contact with the solvent manufacturer, certificates of analyses, or information on the container of the DMF sample. For comparison, different grades of newer DMF samples were also used.

Example: a3–15 = Aged DMF treated by sparging with nitrogen gas for 15 minutes.

2.1 | General methods for treating aged DMF (see Table 3):

2.1.1 | Treatments 1 and 2: Basic alumina or silica method

Basic alumina or silica gel (1.0-1.5 g) was placed in a chromatography column. Aged DMF (50 mL) was passed through the basic alumina or silica gel and was collected by gravity in an amber bottle that has been flushed four times with N_2 for 5 seconds each time. The aged DMF was passed through basic alumina or silica gel one more time, using

TABLE 2 Summary of the DMF used during the studies

	Grade and percentage purity	Manufactured on:	Opened on:
Aged DMF	Grade not specified, ≥99.5%	Feb 2016	July 2016[||]
Newer DMF	Reagent grade, ≥99.0%	Feb 2017	April 2018[‡]
	HPLC grade, ≥99.9%	Feb 2017	June 2018[‡]
	Peptide synthesis grade, ≥99.8%	Dec 2017	June 2018[‡]
	Extra dry grade, ≥99.8%	May 2018	June 2018[‡]

Abbreviations: DMF, dimethylformamide; HPLC, high performance liquid chromatography. \|\| = Exposed to air many times after opening the sealed bottle. ‡ = Used immediately after opening the sealed bottle and stored under N_2 after use.

TABLE 3 Notation used to describe different DMF samples

Notation	Description of treatments of aged DMF
aN[I]-N[II]	Treated aged DMF where N[I] denotes type of treatment and N[II] denotes duration of treatment in minutes, where applicable
N[1]	
1	Passed through basic alumina
2	Passed through silica gel
3	Sparged with inert gas (N_2)
4	Sonicated under vacuum
5	Passed through basic alumina and then silica gel
6	Sparged with inert gas and then sonicated under vacuum
7	Passed through silica gel, sparged with inert gas and then sonicated under vacuum
8	Passed through basic alumina, sparged with inert gas and and then sonicated under vacuum
	Description of the grades of new DMF
b	New, reagent grade DMF
c	New, HPLC grade DMF
d	New, peptide synthesis grade DMF
e	New, anhydrous grade DMF

Abbreviations: DMF, dimethylformamide; HPLC, high performance liquid chromatography.

a different column with fresh basic alumina or silica gel, collected once more in an amber bottle that has been flushed with N_2 as described above. a1 and a2 were stored under an inert atmosphere and were immediately used for the initial loading experiments.

2.1.2 | Treatment 3: Sparging method

Aged DMF (50 mL) was placed in an amber bottle that has been flushed four times with N_2 for 5 seconds each. A modified silicone tube fitted with a 3-mL syringe and a Luer needle was connected to an N_2 line, and the syringe component of the modified tube was submerged under the aged DMF. The N_2 gas was switched on and the aged DMF was bubbled for 15 or 30 minutes. a3–15 and a3–30 were stored under an inert atmosphere and immediately used for the initial loading experiments.

2.1.3 | Treatment 4: Vacuum sonication method

Aged DMF (50 mL) was placed in an amber bottle that has been flushed with N_2 for 5 seconds each. The bottle was attached to a vacuum pump via an adaptor and the bottle was placed in a sonicator. The vacuum pump and the sonicator was switched on simultaneously and the solvent was vacuum sonicated for 15 or 30 minutes. a4–15 and a4–30 were stored under an inert atmosphere and were immediately used for the initial loading experiment.

2.1.4 | Treatments 5 to 8: Combination of treatments

Combinations of the treatments were also carried out to regenerate aged DMF as described above.

2.2 | General method for the initial loading of Fmoc-Gly-OH onto RA-MBHA resins

In a 2-mL disposable MultiSynTech manual peptide synthesis reactor, Rink Amide-MBHA-PS resin (50 mg, 0.580 mmol.g^{-1} capacity) was added. The resin was swollen in dichloromethane (DCM; 1 mL) and agitated for 15 minutes. The resin-bound Fmoc groups were removed using 96:2:2 DMF:DBU:Piperidine (0.5 mL) with agitation for 15 minutes (new reagent-grade DMF (≥99.0%) was used).[42] The resin was washed with the solvent[†] (1 mL) and then washed eight more times (two alternating washes of 2 × 1 mL solvent[†] and 2 × 1 mL MeOH). Fmoc removal was monitored visually using the qualitative Kaiser test in test tubes.[43] The resins were washed with DCM (2 × 0.5 mL) and then suspended in DCM (1 mL) with agitation for 15 minutes. Fmoc-Gly-OH (3 equiv.) and HCTU (3 equiv.) dissolved in the solvent[†] (0.4 mL) was added to the reactor followed by agitation for 5 minutes. In the case of greener solvents, sonication for up to 20 to 25 minutes was required to form a solution. DIPEA (6 equiv.) dissolved in the solvent[†] (0.1 mL) was added to the reactor followed by agitation for 40 minutes. The coupling reaction was carried out at room temperature. The resin was rewashed with the solvent[†] (1 mL) and then washed eight more times (two alternating washes of 2 × 1 mL solvent[†] and 2 × 1 mL MeOH). The resin was washed two more times with MeOH (1 mL) and the excess MeOH was removed by attaching the reactor to a 5 mL syringe via a Luer-lok connector and pulling the plunger of the second syringe to the 5 mL mark. The resin was dried overnight in vacuo at room temperature in preparation for the spectrophotometric estimation of initial resin loading.

† = Aged DMF, treated DMF, new DMF, GVL, NFM and CYR.

2.3 | General method for the spectrophotometric estimation of resin loading[37,44,45]:

Dry resin was placed carefully into a preweighed 2-mL microtube and the weight of the dry resin was determined by difference (6–15 mg). New reagent grade (≥99.0%) DMF was used during the spectrophotometric estimation of loading. DMF (0.8 mL) was added to the microtube to swell the resin. The microtube was vortexed for

5 seconds and then placed on a microtube rack, which was placed on a gyratory rocker. The microtube rack was gently agitated at 70 rpm for 15 minutes on the gyratory rocker at room temperature. The microtube was vortexed once more for 5 seconds and then piperidine (0.2 mL) was added to generate the DBF-piperidine adduct. The microtube was vortexed twice for 10 seconds to ensure good mixing, and placed back on a microtube rack, which was placed on a gyratory rocker again and gently agitated as previously. The resin was vortexed again for 10 seconds, allowed to settle and three 100-fold dilutions of the supernatant were prepared in 2-mL microtubes, using DMF as the diluent. $A_{290\ nm}$ was obtained (triplicate, $n = 3$) for each of the three supernatant dilutions versus a DMF blank in 1-mL quartz cuvettes (1 cm path length). For one of the 1:100 dilutions, loading (L, in mmol.g^{-1}) was calculated for each replicate measurement of $A_{290\ nm}$, using the formula in Equation 1 and the mean L was determined. The calculations for L and mean L were repeated for the two remaining 1:100 dilutions to obtain a total of three mean L values. Spectrophotometric estimation of resin loading was performed two more times, as described above, to obtain a total of nine (nonuplicate, $n = 9$) mean loading values. The grand mean was calculated and taken as the initial resin loading in mmol.g^{-1}.

$$Loading\ (mmol.g^{-1}) = \frac{A_{290\ nm} \times D \times V}{\varepsilon_{290\ nm} \times m \times \Lambda}, \tag{1}$$

where $A_{290\ nm}$ = absorbance at 290 nm, D = dilution factor (100), V = volume (1 msL), $\varepsilon_{290\ nm}$ = molar absorptivity at 290 nm expressed in mL mmol^{-1} cm^{-1} (6089 mL mmol^{-1} cm^{-1}), m = weight of resins (g) and Λ = path length (1 cm).

3 | RESULTS AND DISCUSSION

3.1 | Comparison of initial resin loading values

To the best of our knowledge, there have not been any comparative studies in the literature on which method of treating aged and partially degraded DMF is the most efficient at DMA removal, and consequently, improved resin loading. As outlined earlier, initial capacity has been identified as a critical parameter in SPPS since the loading level of the first anchored amino acid unit dictates the maximum possible yield of the peptide.[27-29] In the following study, we used the loading of the simplest Fmoc-amino acid, Fmoc-Gly-OH, onto a solid support to probe the effect of using aged DMF treated by different methods for the initial anchoring. We calculated the initial resin loading of Fmoc-Gly-OH on Rink Amide-MBHA-PS resins (0.580 mmol.g^{-1} capacity, 1% crosslinking) by spectrophotometric methods. Fmoc-Gly-OH was anchored onto the solid-support using aged DMF during the washing steps and the highly important coupling step and compared with the loading using treated DMF (a1 to a8) and new DMF samples of different grades (b to e).

Simple methods of regenerating aged DMF were chosen for rapidity and convenience and to reduce costs. For this reason, distillation of aged DMF was not performed.[46] Aged DMF was treated using simple methods such as passing through silica gel to scavenge DMA (a2). As mentioned in the introductory section, sparging-aged DMF

with an inert gas has been recommended in the literature for treating partially degraded DMF. We also chose sparging with N_2 (a3) as a method of treatment. To investigate whether the presence of FA in aged DMF, which may lead to unwanted formylation reactions or premature resin cleavage, could affect the loading measurements, aged DMF was also treated by passing through basic alumina to scavenge FA (a1) but not DMA. Sonication under reduced pressure is a common technique for degassing solvents for use in high performance liquid chromatography (HPLC).[47] Aside from sparging, we also attempted sonication under reduced pressure as a possible way of removing dissolved DMA gas in aged DMF (a4). Combinations of treatments (a5 to a8) were also performed. Like the new DMF samples used in this experiment, aged DMF was noted to be clear and colourless but had a potent fishy smell compared with the new DMF samples.

DCM, which is a known high-swelling solvent for PS-based resins, was used for the swelling steps.[48] For the removal of the resin-bound Fmoc group of RA-MBHA resins before the attachment of Fmoc-Gly-OH, 96:2:2 DMF:DBU:Piperidine was the chosen solution for deprotection.[42] With this method of deprotection, we found an average of 89% Fmoc removal, associated with qualitative Kaiser test results (a deep purple-ultramarine colour) ($n = 9$). The initial loading strategy chosen involved the stand-alone coupling reagent HCTU and Hünig's base (DIPEA).[49]

Photometry-based estimations of resin loading involving the generation of the DBF-piperidine adduct from the resin-bound Fmoc group is well-documented in the literature; it is a standard and one of the most common methods of evaluating resin loading.[50,51] The DBF-piperidine adduct has absorption maxima at around 267 nm, 290 nm, and 301 nm, with spectrophotometric determinations of resin loading generally obtained at around 290 nm and 301 nm. In the study presented, determinations were performed at 290 nm, because substitution determination at this wavelength has been recently described by Eissler et al as more reliable.[37,44] The results of the comparative loading experiments are shown in Table 4 and Figure 2. Most loading measurements were found to be within the common loading levels of 0.3 to 0.5 mmol.g^{-1} and loading percentages of 50% to 70%, which are deemed as acceptable loading levels.[28,52]

From a data analysis perspective, we had to address the issue of large spread observed during some experiments. For the sets of data, we found that the spread, in terms of the coefficient of variation (CV [%]), ranged between 1.8% and 18.5%. Possible data outlier detection was performed using the robust regression and outlier removal (ROUT) method (Q = 1%) and Grubbs' test (α = 0.05).[53] In all cases, no significant outliers were detected using both methods, despite the relatively large spread observed in some cases.[54]

As outlined earlier, it has been well-documented in the literature that DMF degrades to DMA and FA via hydrolysis and the presence of traces of DMA can result in Fmoc-amino deprotections.[17-19] As shown above, there is strong statistical evidence that there is a significant difference in mean initial resin loading values due to the solvent used during the attachment of Fmoc-Gly-OH onto the resin. The measured resin loading when aged DMF was used during the attachment of Fmoc-Gly-OH was significantly lower ($p \leq 0.0001$) than when new DMF samples (b to e) were used (Table 4 and Figure 2). Interestingly, we also noted that the spectrophotometrically measured initial

TABLE 4 Mean (n = 9) initial resin loading (mmol G^{-1}) values for the loading of Fmoc-Gly-OH onto rink amide-MBHA-PS resins (0.580 mmol G^{-1} capacity) when aged DMF was used during amino acid attachment versus DMF[b] to DMF[e] and DMF[a1] to DMF[a8]. ± denote 95% confidence intervals (CI) and sample standard deviations (s) are in brackets. Percent coefficient of variation = CV (%). Descriptive statistical (DS) analysis was performed using the PRISM 6.0 software

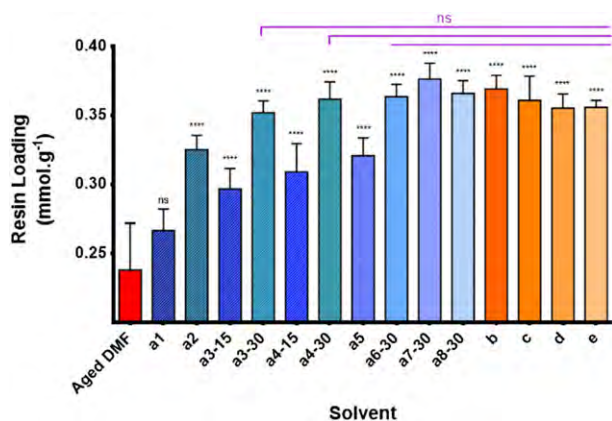|	Fmoc-Gly-OH resin loading (mmol.g^{-1})	CV (%)
Aged DMF	0.238 ± 0.034 (0.044)	18.5
a1	0.267 ± 0.015 (0.020)	7.4
a2	0.325 ± 0.010 (0.014)	4.2
a3–15	0.297 ± 0.015 (0.019)	6.5
a3–30	0.352 ± 0.009 (0.011)	3.1
a4–15	0.309 ± 0.021 (0.027)	8.7
a4–30	0.362 ± 0.013 (0.016)	4.5
a5	0.321 ± 0.013 (0.017)	5.2
a6–30	0.363 ± 0.009 (0.012)	3.2
a7–30	0.376 ± 0.011 (0.015)	4.0
a8–30	0.366 ± 0.009 (0.012)	3.3
b	0.369 ± 0.010 (0.013)	3.5
c	0.361 ± 0.018 (0.023)	6.3
d	0.355 ± 0.010 (0.013)	3.8
e	0.356 ± 0.005 (0.007)	1.8

FIGURE 2 Comparison of the loading in mmol.G^{-1} (n = 9) of Fmoc-Gly-OH onto rink amide-MBHA-PS resins (0.580 mmol.G^{-1} capacity) in aged dimethylformamide (DMF), treated aged DMF and new DMF samples. Loading estimations were based on dibenzofulvene (DBF)–piperidine adduct formation during the removal of resin-bound fluorenylmethyloxycarbonyl (Fmoc) groups. Error bars denote 95% CI. Ns denotes $p > 0.05$ (not significant) and **** denotes $p < 0.0001$ (extremely significant) relative to aged DMF. *P*-values were calculated by univariate analysis (UVA) with a one-way analysis of variance (ANOVA) with Tukey–Kramer's test (post hoc) using the PRISM 6.0 software, where α = 0.05. The purple horizontal lines indicate no statistically significant difference between a3–30, a4–30, a6–30, a7–30, a8–30, b, c, d, and e

resin loading values, when newer DMF samples of different grades were used during the attachment to the resin were not significantly different from each other despite some being older than the others or opened on slightly different dates (ns, Tukey–Kramer test). We also found that vortexing Fmoc-Gly-OH in an aged DMF sample even for just 5 minutes resulted in a small and significant (unpaired t-test, $p < 0.05$) decrease in Fmoc-Gly-OH (%), by UPLC and based on peak areas, compared with vortexing in d, which averaged at 2.1% (n = 5).

When using treated DMF, the measured values improved, with the exception of basic alumina-treated aged DMF (a1). The mean loading when a1 was used was higher than when aged DMF was used. However, our results indicate that the scavenging of FA did not contribute to a statistically significant improvement in loading value. Using aged DMF treated for just 15 minutes resulted in a significant ($p \leq 0.0001$) improvement in the measured resin loading. The initial loading of Fmoc-Gly-Rink-MBHA-PS resins using DMF with longer treatment times was also calculated. When treatment times were extended from 15 minutes to 30 minutes, we observed an even greater improvement in loading levels, which is similar to that of newer DMF samples, as reflected by the mean loading values (ns, Tukey–Kramer test). We found that the loading values when using sparge-aged DMF (a3–30) and sonicated under vacuum (a4–30), each for 30 minutes, did not significantly differ from each other and to newer DMF (b to e). However, it is important to mention that DMF is also known to be subjected to thermal degradation to DMA and carbon monoxide and since sonication can heat samples, care must be taken when choosing this method of DMF treatment.[20]

We also noted that the resin-loading values obtained when aged DMF samples, that underwent a combination of treatments (a5 to a8), were used during the attachment of Fmoc-Gly-OH, did not significantly differ to aged DMF that had been sparged with N_2 (a3–30) or vacuum sonicated (a4–30) for 30 minutes. As such, we deem it unnecessary to carry out combinations of treatments, and that sparging with an inert gas or vacuum sonication would suffice. However, it is important to note that the duration of the treatment time would likely vary and be highly dependent on the age, quality, and quantity of the DMF sample.[18]

As described in the introduction, there has been a surge in interest in implementing green chemistry principles on SPPS in recent years, now termed greener solid-phase peptide synthesis (GSPPS).[9] In line with the 5th principle of green chemistry and for comparison, we also attached Fmoc-Gly-OH onto RA-MBHA resins using greener solvents. Greener solvents chosen in this part of the study include *N*-formylmorpholine (NFM), cyrene (CYR) and γ-valerolactone (GVL). Both NFM and GVL have been proposed as alternatives to DMF for GSPPS by Albericio et al.[6] CYR is a bioderived solvent that has been described as a green dipolar aprotic solvent by Clark et al.[55] Since alternative solvents to DMF are used, unwanted Fmoc removal by DMA is avoided. However, the ring-opening of GVL by amines is of concern. Studies on ring opening of GVL in a SPPS context under certain stress conditions has been recently reported by Albericio et al.[14] Despite this, successful GSPPS of "difficult" peptides using GVL has also been reported, also by Albericio et al.[6]

As shown in Table 5 and Figure 3, we observed that compared with aged DMF, the initial loading of Fmoc-Gly-OH onto the RA-MBHA resin is significantly higher ($p < 0.05$) when greener solvent systems were used, with the exception of CYR. However, in

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TABLE 5 Mean (n = 9) initial resin loading (mmol.g^{-1}) values for the loading of Fmoc-Gly-OH onto Rink Amide-MBHA-PS resins (0.580 mmol.g^{-1} capacity) when aged DMF was used during amino acid attachment versus greener solvent systems. ± denote 95% confidence intervals (CI) and sample standard deviations (s) are in brackets. Percent coefficient of variation (CV [%]). Descriptive statistical (DS) analysis was performed using the PRISM 6.0 software. Data for d from Table 4 is also shown for comparison

	Fmoc-Gly-OH resin loading (mmol.g^{-1})	CV (%)
Aged DMF	0.238 ± 0.034 (0.044)	18.5
GVL	0.327 ± 0.017 (0.022)	6.7
NFM	0.325 ± 0.005 (0.006)	1.8
CYR	0.234 ± 0.031 (0.040)	17.0
d	0.355 ± 0.010 (0.013)	3.8

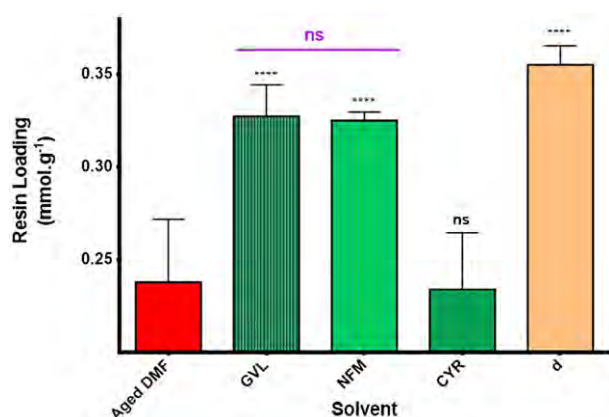Abbreviations: CYR, cyrene; DMF, dimethylformamide; GVL, γ-valerolactone; NFM, N-formylmorpholine.

FIGURE 3 Comparison of the loading in mmol g^{-1} (n = 9) of Fmoc-Gly-OH onto Rink Amide-MBHA-PS resins (0.580 mmol g^{-1} capacity) in aged dimethylformamide (DMF), and greener solvents. Loading estimations were based on dibenzofulvene (DBF)–piperidine adduct formation during the removal of resin-bound Fmoc groups. Error bars denote 95% CI. ns denotes $p > 0.05$ (not significant), ** denotes $p = 0.001$ to 0.01 (very significant) and **** denotes $p < 0.0001$ (extremely significant) relative to aged DMF. p-values were calculated by univariate analysis (UVA) with a one-way analysis of variance (ANOVA) with Tukey–Kramer's test (post hoc) using the PRISM 6.0 software, where α = 0.05. Data for d from Figure 2 is also shown for comparison

comparison with the greener solvents, mean initial loading values were higher when newer DMF samples were used. These greener solvent systems also required an additional sonication step to dissolve HCTU.

3.2 | Testing the quality of aged DMF and treated aged DMF

Two simple methods were selected for evaluating the quality of the DMF samples, namely the DNFB test and refractometry. The DNFB test has been described in the literature as a rapid means of assessing the quality of DMF and detecting the presence of dissolved DMA gas.[18,26,56] DMA content of aged and treated DMF was assessed using the colourimetric DNFB test accordingly.[18] The absorption maximum of the chromophore was determined to be 452 nm in our laboratory.

A$_{452\ nm}$ was obtained (quintuplicate, n = 5) for the aged and treated DMF samples, and the mean of the absorbance values was used to assess whether these DMF samples pass the DNFB test or not. In all cases, all of the treated aged DMF samples passed the DNFB test, signifying that the DMA content is low. Surprisingly, even the aged DMF sample also passed the DNFB test. It indicated that the DMA content of the latter samples were at an acceptable concentration. Although aged DMF also passed the DNFB test, it was noted that the mean absorbance value was highest in aged DMF, indicating greater quantities of DMA compared with treated DMF (A $_{Aged\ DMF}$ > A $_{Treated\ DMF}$). This is consistent with the lower values of the loading achieved when aged DMF was used during the attachment of Fmoc-Gly-OH.

To further test the quality of the aged DMF and treated DMF samples, we measured their refractive indices (RI; n^{20}$_D$; septuplicate, n = 7) and compared the n^{20}$_D$ values with the values obtained for newer DMF samples. Refractometry is an established technique for the rapid assessment of the quality and purity of liquid organic samples, since impurities can lead to RI values different to that of a purer sample. Compared with other methods such as GC, refractometry is a rapid and generally inexpensive test for gaining an insight on the purity of a liquid sample.[57] Nevertheless, its disadvantage as an analytical technique is that it cannot distinguish between the different impurities that may be present in a sample. As previously mentioned, FA has been described in the literature to form alongside DMA during the degradation of DMF. Solutions of FA in reagent grade (≥99.0%) DMF (b) over a broad concentration range were prepared to assess the impact of concentration of FA on the RI of DMF. For reference, the RI of reagent grade DMF (b) was also obtained. Since DMA is a gas, we deemed it impractical to attempt to make solutions of DMA in DMF.

At very low concentrations of FA in DMF, we found that the mean RI values were slightly lower than pure b, but we found that the difference was not statistically significant (ns, Tukey–Kramer test, Figure 4). However, from 1.3×10^{-2} mol/L, we began to observe a statistically significant decrease in RI values as the concentration of FA increased. Between 1.3×10^{-2} M and 2.7×10^{-1} M, the RI values were not significantly different to each other, but were statistically significantly different to the RI of newer reagent grade DMF. After establishing that FA impurities can lead to a statistically significant decrease in the RI of pure DMF, and thus may be useful in evaluating the purity of DMF samples, we measured the RI of all our DMF samples.

We observed that the RI value of our aged DMF was slightly, but significantly ($p < 0.05$), different to the RI values of the newer DMF samples (Figure 5). The lowered RI value of the aged DMF compared with newer DMF samples is likely due to degradation impurities. The low magnitude of the difference in RI signifies that the quantity of impurities in aged DMF is low, which is consistent with what we found using the DNFB test. In most cases, the difference in RI values between aged and new DMF samples were not statistically significant, though there were some exceptions. Interestingly, even though we observed a significant improvement in initial resin loading when a2 and a5 were used during the attachment of Fmoc-Gly-OH onto the resin, the RI values of the a2 and a5 treated samples were not significantly different to the RI value of aged DMF (ns, Tukey–Kramer test).
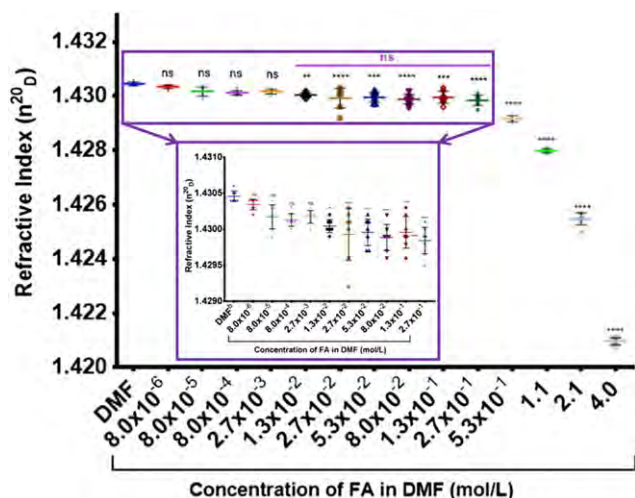
FIGURE 4   Interval plot of the effect of increasing the concentration formic acid (FA) in reagent grade ($\geq$99.0%) dimethylformamide (DMF) (b) on the refractive index. N = 7. Error bars denote 95% CI. Ns denotes $p > 0.05$ (not significant), ** denotes $p = 0.001$ to 0.01 (very significant), *** denotes $p = 0.0001$ to 0.001 (extremely significant) and **** denotes $p < 0.0001$ (extremely significant) relative to reagent grade DMF (b). p-values were calculated by univariate analysis (UVA) with a one-way analysis of variance (ANOVA) with Tukey–Kramer's test (post hoc) using the PRISM 6.0 software, where $\alpha = 0.05$. The refractive index (RI) values for DMF[b] and the FA/DMF solutions up to 2.7×10^{-1} M have been magnified
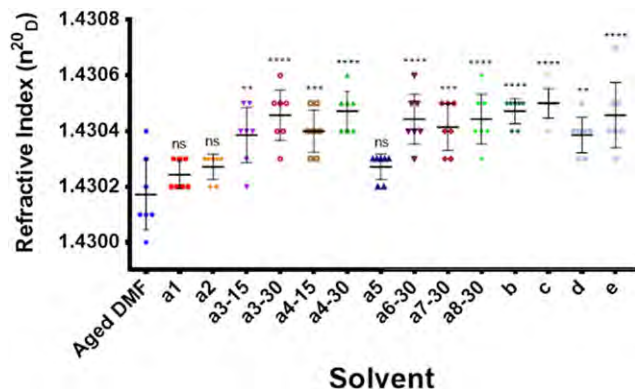

FIGURE 5   Interval plot of the refractive index (RI) values for the different dimethylformamide (DMF) samples. N = 7. Error bars denote 95% CI. Ns denotes $p > 0.05$ (not significant), ** denotes $p = 0.001$ to 0.01 (very significant), *** denotes $p = 0.0001$ to 0.001 (extremely significant) and **** denotes $p < 0.0001$ (extremely significant) relative to reagent grade DMF (b). p-values were calculated by univariate analysis (UVA), with a one-way analysis of variance (ANOVA) with Tukey–Kramer's test (post hoc) using the PRISM 6.0 software, where $\alpha = 0.05$

The RI value for a1 was also not significantly different to that of aged DMF.

In summary, the analytical studies (DNFB test, statistical analysis, and RI measurements) indicate that the quantity of degradation impurities in our aged DMF sample is low, but has contributed to a significant decrease in the measured initial resin loading, when using

the spectrophotometric method based on DBF-piperidine adduct generation for estimating loading. We found sparging with N_2 and sonication under vacuum to be effective in improving the measured loading values.

4 | CONCLUSIONS

We emphasised the importance of the initial loading step in SPPS and the impact of DMF degradation to DMA on Fmoc-SPPS. The spectrophotometrical method of estimating resin loading based on the quantification of the DBF-piperidine adduct is one of the most common and standard methods of assessing loading efficiencies. Our results show that the use of aged DMF for the initial loading step of Fmoc-SPPS can lead to lower calculated initial resin-loading values. Loading significantly improved when aged DMF that has been regenerated, by simple means of treatment, such as sparging with an inert gas and sonication under vacuum, was used for attaching Fmoc-Gly-OH onto the resin. It is also highly likely that treatment times would vary depending on the volume, age, and quality of the DMF sample being used.

Treating aged DMF may be sufficient to reduce the quantity of degradation impurities and hence improve the resin loading values. Where possible, the use of newer DMF samples is also strongly recommended when carrying out Fmoc-SPPS. Despite passing the DNFB test, the mean loading value when aged DMF was used was comparably lower versus newer DMF samples. During our investigation, analytical studies indicated that the quantity of impurities in our aged DMF sample was low, but sufficient enough to lead to less accurate and a significantly lower initial resin loading value.

Alternatively, one may use one of the alternative greener solvents in the published literature, such as GVL and NFM, proposed instead of DMF to totally avoid the issues associated with DMA. However, along with the lower loading levels obtained, the use of greener solvents also required an additional sonication step to dissolve the coupling reagent HCTU.

Although the study of the initial loading step is critically important, the use of DMF for the further Fmoc amino acid coupling steps is of no less importance. As such studies are underway on examining the effects of aged, treated, and newer DMF samples when used in a full SPPS protocol. The results of these studies will be reported in due course.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Exhibit 37

🏴 KeyCite Yellow Flag - Negative Treatment

Declined to Extend by   Good v. American Water Works Company, Inc.,
S.D.W.Va.,   October 13, 2016

2004 WL 1068805
Only the Westlaw citation is currently available.
United States District Court,
E.D. Pennsylvania.

Ryan WESTLEY

v.

ECOLAB, INC., et al.

No. Civ.A.03–CV–1372.
|
May 12, 2004.

Attorneys and Law Firms

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MEMORANDUM & ORDER

SURRICK, J.

 *1 Presently before the Court is Defendant, Ecolab, Inc.'s Motion to Exclude the Testimony of Dr. Michael J. Coyer (Doc. No. 22), Motion to Exclude the Testimony of Dr. Burton Z. Davidson (Doc. No. 23), and Motion for Summary Judgment (Doc. No. 24). For the following reasons each of Defendant's Motions will be denied.

Background

This case concerns the cause of severe burns sustained by Plaintiff, Ryan Westley while cleaning the kitchen floor at Shoobies Restaurant in Sea Isle City, New Jersey. On July 25, 2001, Plaintiff was preparing to clean the floor at Shoobies using Oasis 115XP, a cleaning solution manufactured by Defendant. (Compl.¶ 10.) The cleaning product was dispensed from a dispenser called the Oasis II, which mixed water with the cleaning product to form a solution. (*Id.* ¶ 11.) The dispenser is designed to allow the user to push a button that mixes concentrated 115XP with water

in a bucket. The concentration of the solution can be changed based on how the dispenser is installed. In order to fill a mop bucket with solution, Plaintiff placed a hose connected to the dispenser and the hot water heater, into a bucket, and then pressed the button on the dispenser to mix the water with the cleaning product which flowed into the bucket. (*Id.* ¶ 12.) Plaintiff left the hose in the bucket, allowing it to fill with solution, and left the area to complete other tasks. Upon returning to the bucket, Plaintiff found the bucket almost completely filled to the top. Recognizing that solution would spill on the floor if a mop was placed in the bucket, Plaintiff lifted the bucket up to the sink and began pouring some of the solution into the sink. (*Id.* ¶ 16.) During the process of pouring the solution into the sink, some of the solution spilled onto Plaintiff's thighs, lower legs, ankles, and feet. Plaintiff did not feel any immediate pain and he began mopping the floor. Within four to five minutes, Plaintiff felt a horrible burning on his feet and ankles. (*Id.* ¶ 19.) Plaintiff removed his shoes and socks and began to douse the affected areas in cold water. The skin on his feet and ankles had already begun to peel and blister. (*Id.* ¶ 20.)

An ambulance was called and Plaintiff was taken to Burdette Tomlin Memorial Hospital. Plaintiff was then transferred, via ambulance, to the burn unit of St. Agnes Medical Center. At the hospital the damaged skin was debrided. The next day, doctors in the burn unit performed a skin graft. The doctors removed skin from Plaintiff's upper thighs and grafted it to his ankles and feet. (*Id.* ¶ 27.) Plaintiff was hospitalized for approximately ten days. (*Id.* ¶ 28.) As a direct result of this incident, Plaintiff sustained injury and received treatment as follows: second and third degree burns of both feet and ankles; surgery involving excision of burned tissue and skin grafts, permanent scarring measuring 15 x 13.5 centimeters at the site where skin was taken from Plaintiff's upper thigh; permanent scarring measuring 13.5 x 6.5 centimeters upon the medial aspect of Plaintiff's right foot; permanent scarring measuring 12 x 4.5 centimeters upon the medial aspect of Plaintiff's left foot; permanent hypertrophy and pigment changes to the affected areas; and permanent itching of the affected areas.

 *2 Defendant is in the business of designing, manufacturing, marketing and selling the cleaning product known as Oasis 115XP, and the mechanism for dispensing Oasis 115XP called the Simplex Dispenser ("dispenser"). [1] (*Id.* ¶ 5.) In the course of its business, Defendant sold and installed a dispenser for the Oasis 115XP product, which was being used at Shoobies on July 25, 2001.

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On January 28, 2003, Plaintiff filed this lawsuit against Defendant in the Court of Common Pleas of Philadelphia County alleging negligence and strict liability. On March 3, 2003, the matter was removed to this Court. Plaintiff asserts that the product manufactured by Defendant caused his injuries. Plaintiff contends that "Defendant had the duty to adequately and reasonably advise and instruct the foreseeable users on the safe and proper use of products and warn foreseeable users of the dangers associated with the use of the products." (*Id.* ¶ 35.) Plaintiff contends that Defendant breached this duty, causing the injury that Plaintiff sustained. Alternatively, Plaintiff contends that Defendant's products, used by Plaintiff, were "not reasonably fit, suitable or safe for their intended purposes, and were in a defective condition and unreasonably dangerous to the user or consumer." (*Id.* ¶ 43.) Defendant contends that the Oasis 115XP use solution did not cause Plaintiff's injuries. Defendant contends that testing of its product demonstrated that it could not caused these burns. Defendant points to several of the medical records and argues that Plaintiff's injuries are consistent with thermal burns caused by boiling or scalding water.

In support of the argument that Defendant's products caused Plaintiff's injuries, Plaintiff intends to offer the expert testimony of Dr. Michael J. Coyer and Dr. Burton A. Davidson. Defendant contends that this expert testimony should be excluded under Fed.R.Evid. 702 and *Daubert v. Merrell Dow Pharm., Inc.,* 509 U.S. 579, 113 S.Ct. 2786, 125 L.Ed.2d 469 (1993). Defendant also contends that because this expert testimony is not admissible, we should grant summary judgment in favor of Defendant, there being no issue of material fact. FED. R. CIV. P. 56(c). Plaintiff argues that both experts are qualified, and that their testimony will assist the trier of fact.

Discussion

Under the Federal Rules of Evidence, the trial judge must ensure that any and all scientific testimony or evidence is not only relevant but reliable. *Daubert,* 509 U.S. at 590. Specifically, Rule 702 provides that:

> If scientific, technical or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a

> witness qualified as an expert by knowledge, skill, experience training, or education, may testify thereto in the form of an opinion or otherwise, if (1) the testimony is based upon sufficient facts or data; (2) the testimony is the product of reliable principles and methods; and (3) the witness has applied the principles and methods reliably to the facts of the case.

*3 FED.R.EVID. 702. In *Daubert,* the Supreme Court said that determining whether the expert is proposing to testify to scientific knowledge that will assist the trier of fact involves a "preliminary assessment of whether the reasoning or methodology underlying the testimony is scientifically valid and of whether that reasoning or methodology properly can be applied to the facts in this case." 509 U.S. at 592–93.

In applying *Daubert,* the Third Circuit instructs that Rule 702 requires a district court to assess an expert's "qualifications" and the "reliability" of the proposed testimony. *Oddi v. Ford Motor Co.,* 234 F.3d 136, 145 (3d Cir.2000); *In re Paoli Railroad Yard PCB Litig.,* 35 F.3d 717 (3d Cir.1994). In assessing the "reliability" of the testimony, the factors to be considered are:

> (1) whether a method consists of a testable hypothesis; (2) whether the method has been subjected to peer review; (3) the known or potential rate of error; (4) the existence and maintenance of standards controlling the technique's operation; (5) whether the method is generally accepted; (6) the relationship of the technique to methods which have been established to be reliable; (7) the qualifications of the expert witness testifying based on the methodology; and (8) the non-judicial uses to which the method has been put.

Id. (citing 🚩*Paoli,* 35 F.3d at 742 n. 8). "The test of admissibility is not whether a particular scientific opinion has the best foundation or whether it is demonstrably correct. Rather, the test is whether 'the particular opinion is based on valid reasoning and reliable methodology." ' 🚩*Oddi,* 234 F.3d at 145–46 (quoting 🚩*Kannankeril v. Terminix Int'l Inc.,* 128 F.3d 802, 806 (3d Cir.1997). In addition, even if the expert's methodology is found to be sufficient, to be admissible the testimony must also be found to assist the trier of fact. 🚩*Paoli,* 35 F.3d at 743.

In making a determination as to whether an expert is qualified to give testimony, the Third Circuit has stated that: "Rule 702 requires the witness to have 'specialized knowledge' regarding the area of testimony.... '[A]t a minimum, a proffered expert witness ... must possess skill or knowledge greater than the average laymen." ' 🚩*Elcock v. Kmart Corp.,* 233 F.3d 734, 741 (3d Cir.2000) (quoting 🚩*Waldorf v. Shuta,* 142 F.3d 601, 625 (3d Cir.1998)); *see also* 🚩*Holbrook v. Lykes Bros. Steamship Co.,* 80 F.3d 777, 782 (3d Cir.1996) (holding that "it is an abuse of discretion to exclude testimony simply because the trial court does not deem the proposed expert to be the best qualified or because the proposed expert does not have the specialization that the court considers most appropriate").

Testimony of Dr. Coyer
Plaintiff seeks to offer the testimony of Dr. Michael J. Coyer who will testify as to the cause of Plaintiff's injuries. Defendant contends that the testimony of Dr. Coyer is inadmissable because he is unqualified, and because the methodology underlying his testimony is unreliable. We will first assess the adequacy of Dr. Coyer's qualifications.

Dr. Coyer's Qualifications
 *4 Dr. Coyer intends to offer his opinion regarding Plaintiff's exposure to the 115XP solution and whether the exposure caused Plaintiff's burns. Plaintiff contends that Dr. Coyer is qualified to offer testimony on these "classic toxicology and chemistry opinions ." (Doc. No. 28 at 25.) From 1982 through 1992, Coyer studied physical chemistry, organic chemistry, and analytical chemistry at the University of Scranton, the University of Oklahoma, and the State University of New Jersey – Rutgers. (Doc. No. 22 Ex. E ("Coyer Report")

at 5.) During this time, he received a masters degree and a Ph.D. in inorganic and physical chemistry. For the past fourteen years, Dr. Coyer has worked in the fields of forensic chemistry and toxicology. He is presently employed by Clinical Laboratories, Inc., where he supervises a forensic and toxicology laboratory. Prior to his current employment he was the director of Sierra Analytical Laboratories, another forensic toxicological laboratory. (*Id.*)

Dr. Coyer is a member of the Society of Forensic Toxicology and is certified by the American Board of Forensic Toxicology for Diplomate status. [2] (*Id.*) In addition to working in the private sector, from 1996 to 2000, he served on the Adjunct Faculty at Marywood College, teaching courses in Forensic Science and Environmental Monitoring. (Coyer Report at 5.) In addition, he has also authored six published articles within the fields of chemistry and toxicology. (*Id.* at 6.)

Defendant contends that although Dr. Coyer is a toxicologist, he is not qualified to give testimony in this instance because he has no educational or "real-life" experience relating to the effects of chemicals on human tissue. (Doc. No. 22 at 13.) Dr. Coyer admits that he has no experience dealing with burns on living people, has not taught a class or published an article concerning burns or industrial cleaning products, or performed any studies on potassium or ammonium hydroxide (the two potentially caustic agents in Oasis 115XP). (Coyer Dep. at 37, 43–45.) This does not disqualify Dr. Coyer from offering his opinion in this matter. Rather it goes to the weight to be accorded to his testimony by the fact finder. Dr. Coyer has vast knowledge and experience regarding toxic chemicals, including potassium hydroxide and sodium hydroxide, which he characterizes as "very common chemicals." (Coyer Dep. at 44.) Based on Dr. Coyer's wide range of experience in chemistry and toxicology we conclude that he has satisfied the "liberal" requirements necessary to testify as an expert in this matter. *See* 🚩*Paoli,* 35 F.3d at 741.

Dr. Coyer's Opinions
Dr. Coyer has submitted a report containing his opinion concerning Plaintiff's injuries. In this report he concludes that "the injury to Mr. Westley ... [is] consistent with chemical burns resulting from an aqueous solution of at least 2 ounces per gallon of Ecolab 115XP." (Coyer Report at 1.) This opinion is primarily based upon Dr. Coyer's knowledge of the

active chemicals in Ecolab 115XP and the effects of these chemicals. [3] His opinion is as follows:

 *5 The materials referred to, ammonium hydroxide and potassium hydroxide, are those 'active ingredients' found in Ecolab product 115XP. As a characteristic of chemical burns, the responsible agent will continue a destructive path until it is either removed or completely neutralized. (1) In the discussion of the hydroxide associated with this case, one must refer to the pH of the solution. These hydroxide materials characteristically have pH values above neutral (pH (neutral) = 7.0 Scale 1–14 units) and are categorized as 'basic' or 'alkaline.' By definition, a pH value below 7.0 is considered 'acidic.' The remainder of this discussion, based on the circumstances surrounding this case, will be limited to these 'basic/alkaline' properties of the chemicals in question.

  1. The standard initial treatment for chemical burns is hydrotherapy (i.e. the irrigation of the effected area with large amounts of water.) This course of action dilutes the area of the causative agents until further treatment is available. It is well known that alkali materials reaction with water is exothermic (i.e. generate heat). (2) The hydrotherapy can keep heat production to a minimum.

     It has been shown in experimental animals that with alkali burns, the pH of burned tissue does not return to normal for up to twelve (12) hours even with initial and continuous hydrotherapy. This extended delay to a return to normal pH is due to the chemical reaction of the hydroxides with components of tissue (i.e.proteins, lipids, etc.) forming complexes that facilitate the movement of hydroxyl ions into deep tissue. This movement into deeper tissues provides a difficult gradient barrier for the hydrotherapy to effectively contact with the agent. This is a characteristic of an alkahi burn, which requires the extensive and continued hydrotherapy to bring to a normal pH. (1)

  2. In contrast to acids, alkalis do not coagulate protein, which could impede penetration of the compounds. The hydroxide burns may not be immediately painful while the material penetrates the skin. The contact with skin, even by dilute solutions, can cause severe and slow healing burns. The extent of injury is usually a factor of contact time and solution strength. (3)

3. In the case of Mr. Westley, his clothing, specifically his footwear, was saturated with the 115XP mixture. According to his statement, the contact time before removal of the footwear was about 4–5 minutes. At that time, he began a form of hydrotherapy. This delay would have played a significant role in the damage depicted in the accompanying photographs. The reaction with 115XP solution and consequential damage to the skin tissue was initiated during the immediate contact time. In that time, the characteristic alkali reaction had begun a course, eventually leading to the described injuries. As mentioned previously, the pH of this solution would not have significantly been reduced to normal upon the induction of the hydrotherapy.

 *6 4. The Law of Mass Action deals with equilibrium at a given temperature. It is that temperature at which certain characteristics of substance can be monitored and predictions of reaction rates, solubility, etc. can be postulated. In this case, the water temperature of the solution in question was stated to be about Ĩ30 degrees F. This was an estimate of water temperature extrapolated from information in these listed depositions. The fact of this case is that the water was either 'warm' or 'hot.' The elevated water temperature would have played a role in the tissue damage of Mr. Westley in several ways:

a. The higher temperature itself may have weakened the tissue or changed the porosity of the skin to allow the alkali metal solution to readily enter the tissue system.

b. The solubility of the chemicals of the solution would have increased making more alkali materials (i.e.hydroxides) available to the effects of absorption. (3)

....

In summary, I can conclude within a reasonable degree of scientific certainty that the wounds suffered by the Plaintiff, Ryan Westley, were directly related to his 25, July, 2001 exposure to the aqueous 115XP. The basis of my conclusions are the information from the documents and information presented to me in this case, the pH measurements of the various concentrations of solutions, the chemical burn references and the material safety data sheets used in the preparation of this report. It is those characteristics of alkali materials, described in

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the text of this report and the fact that the solution was at some elevated temperature, that I draw this scientific conclusion. My opinion, is also based on both generally accepted chemical properties and those references published with regards to chemical alkali burns.

(Coyler Report at 2–3.)

Defendant argues that based on Dr. Coyer's reports and deposition testimony, the methodology behind his opinion does not meet the *Daubert/Paoli* standard of reliability. First, Defendant contends that Dr. Coyer's testimony does not follow any generally accepted methodologies. Specifically, Defendant finds the opinion lacking because "[Dr. Coyer] reaches his conclusion by simply relying on Plaintiff's version of the truth, without citing any testing or peer-reviewed materials to support his theory." (Doc. No. 22 at 18.) Defendant argues that tests it conducted show that 115XP could not have caused Plaintiff's injuries. (Doc. No. 25 G, H.) Defendant proffers that its tests also support the argument that Dr. Coyer's methodology is flawed. Finally, Defendant contends that the opinion is unreliable because Dr. Coyer conducted no tests of his own to establish that the solution could have caused Plaintiff's injuries.

Dr. Coyer's opinion is primarily based on his experience and knowledge of chemicals and specifically his knowledge of aluminum hydroxide and potassium hydroxide and their potential effects on human tissue, as well as the facts related by Plaintiff.[4] Opinions based solely on experience and knowledge are sufficient under *Daubert. See* [flag]*Kannankeril, 128 F.3d at 809* (holding expert testimony admissible where expert "relied on general experience and readings, general medical knowledge, standard textbooks, and standard references"). In formulating his opinion, Dr. Coyer also relied upon Dr. Frederick A. DeClement's medical diagnosis of Plaintiff's injuries. (Coyer Report at 1; Doc. No. 28 Ex. 17.) Dr. DeClement as well as Plaintiff's treating physicians, diagnosed Plaintiff as having suffered "*chemical* burns." (*Id.* (emphasis added).) Dr. Coyer also considered the Material Safety Data Sheets ("MSDS") for the 115XP solution (versions one and two) and 115XP Concentrate. (Doc. No. 28 Exs. 8, 9.) In one of these sheets, Defendant warned about the hazardous nature of potassium hydroxide and ammonium hydroxide when it came in contact with human skin stating: "CAUSES SERIOUS CHEMICAL BURNS".[5] (*Id.*) The other MSDS warned that it "CAUSES CHEMICAL BURNS." (*Id.*)

*7 Defendant argues that the tests it that it conducted illustrate the unreliability of Dr. Coyer's opinion. In *Kannankeril* the Third Circuit addressed the issue of the admissibility of expert testimony that is not supported by individual testing and is contrary to evidence introduced by the opposing party. [flag]*128 F.3d at 807*. The court stated: "Depending on the medical condition at issue and on the clinical information already available, a physician may reach a reliable different diagnosis without himself performing a physical examination, particularly if there are other examination results available." *Id.* Despite the fact that the expert in *Kannankeril* came to a different conclusion, the court held the testimony admissible stating: "[plaintiff's] burden is only to provide an expert opinion that is relevant and reliable and that will assist the trier of fact.... [I]ssues of credibility arise after the determination of admissibility. Credibility is for the jury." *Id.* at 809–10. The same principle applies here. While the tests conducted by Defendant might affect the jury's assessment of the credibility of Dr. Coyer's opinion, the existence of the test results and the seemingly contradictory conclusions do not preclude a determination that the opinion is admissible.

Notwithstanding Defendant's contentions that Dr. Coyer's opinion is flawed because it does not rely on any testing or literature, and does not rule out any other cause of Plaintiff's injuries, we are satisfied that Dr. Coyer's testimony is admissible under *Daubert.* Coyer relied on his general experience, scientific knowledge and medical and scientific reports in forming his opinion. The requirements of *Daubert* have been met.

Testimony of Dr. Davidson

Plaintiff seeks to admit the testimony of Dr. Burton Z. Davidson, who will testify regarding the toxicity of the solution that Plaintiff spilled on his feet, the failures of Defendant related to product warnings, and product design of the dispenser of the 115XP solution. Defendant seeks to exclude Davidson's testimony, based upon the fact that he is unqualified to testify on any of the subjects for which he is being offered, and because the methodology underlying his testimony is unreliable. We will first assess the adequacy of Davidson's qualifications.

Dr. Davidson's Qualifications

Davidson has extensive experience in the areas of chemistry and chemical safety. He attended Syracuse University

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receiving a bachelors and a masters degree in chemical engineering. He then attended Northwestern University where he received a Ph.D. also in chemical engineering. (Doc. No. 22 Ex. P ("Davidson C.V.") at 3.) After receiving his Ph.D., Dr. Davidson began teaching at Rutgers University in the fields of chemical engineering, chemical safety, chemical kinetics, general chemistry, and biochemistry. His tenure as a chemistry professor at Rutgers has extended over forty years, eighteen of which he served as the chair of the chemistry department at Rutgers. (*Id.*) He has also been recognized as a "Distinguished Professor," a designation only given to ten percent of the faculty. (Doc. No. 28 at 47.) Throughout his career, Davidson has worked extensively with caustic chemicals and has done experiments regarding the effect of chemicals on human skin tissue. (Doc. No. 22 Ex. Q ("Davidson Dep.") at 85–86.) Dr. Davidson has been called upon to render his expert opinion in cases involving caustic drain cleaners on many occasions. (*Id.* at 37.)

 *8 Dr. Davidson's current academic focus is chemical kinetics and safety engineering. (*Id.* at 26.) Pursuant to his work in this field, he received Certificates of Designation as Safety, Health & Environmental Ergonomist/Associate and Certified Forensic Ergonomist by the Board of Certified Safety Health & Environmental Ergonomists. (Davidson C.V. at 5.) He is also currently a member of the American Society of Safety Engineers, the American Society of Engineering Education, and the American Institute of Chemical Engineers. (*Id.* at 8.) Finally, Dr. Davidson estimates that since he earned his professional license, he has provided opinions on warnings in more than 125 chemical product cases and other consulting arrangements. (Davidson Dep. at 36–37.)

Defendant contends that Dr. Davidson is not qualified to testify as to the caustic effect of chemicals on human tissue because he has never specifically studied the effects of potassium or ammonium hydroxide on human tissue, nor has he published any materials or done significant work in the toxicology field. (Doc. No. 23 at 10.) As noted above, Dr. Davidson received a Ph.D. in chemical engineering, has taught numerous chemistry and safety-related classes and has done research on the effects of chemicals on human tissue. We are satisfied that Dr. Davidson's experience and education ensure that he is adequately qualified to testify about all of the matters for which he is offered. *See* ⚑ *Paoli,* 35 F.3d at 741.

Dr. Davidson's Opinions: Proximate Cause

In his report, Davidson gives his opinions regarding the proximate cause of Plaintiff's burns as well as the design defects in Defendant's product and the safety warning defects. With respect to the proximate cause of Plaintiff's injury, Davidson opines:

> The above medical opinions of Ryan Westley's chemical burns injuries are completely consistent with universal (and scientifically established) chemical reaction principles, too! That is, the corrosive attack on human tissue by potassium and ammonium hydroxide solutions (at any water dilution concentrations and temperature) follows the Guldberg and Waagl (1867) principle of "mass action". According to this reaction rate principle, the corrosive chemical attack of alkali (potassium and ammonium hydroxide) on human tissue (e.g. fats and collagen) is proportional to the concentrations of the alkali species at constant temperature. Also, the constant of proportionality increases exponentially with increasing temperature according to the well know [sic] Arrhenius principle. In other words, the corrosive attack by alkali is accelerate by elevated temperatures! One of the principal reactions of the corrosive attack of alkali on human tissue is "saponification". Saponification makes "soap" out of human tissue! The chemical attack of alkali on human tissue causes deep corrosion because of the diffusion and reaction processes occurring. Additionally, elevated temperatures (e.g. at, or around, 120–140 degrees [F]ahrenheit) accelerate and deepen the corrosive attack on human tissue.

*9 (Doc. No. 22 Ex. P ("Davidson Report") at 3–4.)

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Defendant contends, that like the opinions of Dr. Coyer, this opinion is unreliable because the opinion is not based upon any generally accepted methodologies, is not supported by testing or literature, and cannot rule out other causes. (Doc. No. 23 at 12–13.) Dr. Davidson's conclusion that the 115XP solution caused Plaintiff's chemical burns is based on many of the same documents and depositions that form the basis of Dr. Coyer's opinion. Specifically, Dr. Davidson considered the various depositions regarding the event, the medical reports related to Plaintiff's injuries and Defendant's documentation related to 115XP solution. (Davidson Report at 1.) He also used his knowledge of the effects of chemicals on human tissue and his knowledge of chemistry and toxicology in applying the Law of Mass Action and the Arrhenius principle to the facts in this case. We are satisfied that under *Daubert/ Paoli,* Dr. Davidson's opinion is reliable. The fact that Dr. Davidson conducted no independent testing of the possible affects of the 115XP solution is not controlling.

Dr. Davidson also gives his opinion concerning how Plaintiff's injury could have been avoided. He opines that Defendant failed to provide proper training for Plaintiff's employer and Plaintiff on how to use the 115XP dispenser, failed to provide proper warning labels and defectively designed the dispenser. Dr. Davidson summarized his opinions as follows:

> In the field of chemical safety engineering, a product and/or process must be reasonably free of all recognized hazards to people, property, and/or environment. In the instant matter, the ultimate end-user (Ryan Westley) was exposed to multiple hazards involving the predictable use of the Green Dispenser use wash solution in a restaurant kitchen setting. The product (i.e. Ecolab's 115XP use wash solution) and the processes (i.e. Ecolab's Green Dispenser operation) were not free of recognized hazards. These hazards, individually and/or in combinations did cause Ryan Westley's serious bodily injuires.

These hazards were

(i) Inappropriate and defective Product Stewardship training (R–1, R–5, R–6, and R–8) between Ecolab and Shoobies restaurant owner. [6]

(ii) Defective MSDS for Oasis 115XP use solution. The section on HEALTH HAZARD DATA for skin contact exposure was defective (R–7 and R–10).

> The sections FIRST AID (skin) aid PROTECTIVE MEASURES (skin) were also defective.

> (iii) No HEALTH HAZARD FIRST AID, and PROTECTIVE MEASURES warnings information on the Green Dispenser, which is the ultimate-point-of-risk (R–8 and R–10).

> (iv) The Green Dispenser's start button and controls were defectively designed (R–8, R–11, and R–12).

(Davidson Report at 4.) In coming to his conclusions that Plaintiff's injuries could have been avoided, Dr. Davidson relied upon Occupational Safety and Health Administration ("OSHA") standards, American National Standards Institute ("ANSI") standards, New Jersey state law, and relevant standards in the chemical manufacturing industry. (Davidson Report at 1, 2.)

Dr. Davidson's Opinions Re: Training and Adequate Warnings

*10 Defendant contends that Dr. Davidson's testimony concerning training and warnings is inadmissible under *Daubert.* First, Defendant argues that the type of testimony Dr. Davidson seeks to give with respect to the inadequate warning is inadmissable. Defendant compares Dr. Davidson's testimony to the expert's opinions that the Eighth Circuit found inadmissible in *Robertson v. Norton Co.,* 148 F.3d 905, 907 (8th Cir.1998). In *Robertson,* the court found that the trial court had improperly allowed an expert to testify on defective product warning labels because the expert was not qualified to testify concerning warning labels, and the expert's opinion was not supported by "the kind of scientific theory, practical knowledge and experience, or empirical research and testing ..." *Id.* The instant case can be distinguished. Not only is Dr. Davidson an expert on caustic chemicals he is also an expert regarding safety. He has taught classes concerning adequate warnings, testified as an expert in cases concerning chemical warnings, and has served as a member of

a warning label design team assembled by the Federal Trade Commission.

Dr. Davidson's opinion regarding Defendant's failure to adequately train and warn of the dangers associated with 115XP is also based on numerous OSHA and ANSI labeling standards. [7] (Davidson Report at 2.) Defendant argues that the OSHA standards underlying Davidson's opinion only apply to Shoobies, Plaintiff's employer. For this reason, Defendant claims that the testimony is inadmissible under *Daubert.* Pursuant to the Occupational Safety and Health Act, ⚑ 29 U.S.C. § 655(a), the Hazard Communication Standard ("HCS") was promulgated to "ensure that the hazards of all chemicals produced or imported are evaluated, and that information concerning their hazards is transmitted to employers and employees." 29 C.F.R. § 1910.1200(a)(1). Defendant argues that *only* Shoobies as Plaintiff's employer had a duty to warn and train its employees about dangerous chemicals. (Doc. No. 23 at 28.) Defendant's argument misconstrues the plain reading of the HCS. While the HCS places duties on employers to protect employees, it also imposes duties on chemical manufacturers. The HCS requires that any hazardous chemicals that leave the place of manufacture be "labeled, tagged, or marked with (i) the identity of the hazardous chemical; (ii) appropriate hazard warnings ..." 29 C.F.R. § 1910.1200(f)(1). In addition, chemical manufacturers must "obtain or develop a material safety data sheet for each hazardous chemical" it produces or imports. 29 C.F.R. § 1910.1200(g)(1). Under these regulations Defendant had a duty to Plaintiff to adequately warn about the dangers associated with 115XP solution.

Dr. Davidson's testimony regarding Defendant's failure to adequately train Shoobies' employees (including Plaintiff) regarding the dangers associated with 115XP solution is based on the notion of "product stewardship." (Davidson Report at 5.) As Dr. Davidson explains: "Product Stewardship (PS) has been a well-recognized and practiced safety tool of the Chemical Manufacturers Association (CMA), and others, for decades. It is part of CMA's Responsible Care doctrine of 1990. One of the key features of the PS is to 'counsel customers on the safe use of chemicals' ". (Davidson Report at 5 (internal citations omitted).) Dr. Davidson states that Defendant did not properly counsel Shoobies (and Plaintiff) on the "necessity to use proper protective gear and access [Shoobies] safety understanding of the MSDSs [sic]." (*Id.*) Dr. Davidson contends that:

> ***11** "a proper PS program, had it been implemented, and audited, would have ensured effective training of Ryan Westley and his employer on ways to avoid skin exposure. Ecolab owned the Dispenser equipment, supplied the hazardous chemicals, maintained and installed the equipment, trained the owner of Shoobies Restaurant, and has a contract with Shoobies Restaurant. Ecolab thus had safety duties to Shoobies' workers and a PS relationship with Joe Roberts."

(*Id.*)

Defendant argues the concept of product stewardship does not apply to this case. Defendant contends that the standards espoused under the theory of product stewardship do not apply because Ecolab is an inherently different chemical manufacturer than Dow Chemical, the company that developed the product stewardship theory. (Doc. No. 23 at 29.) Defendant also argues that Dr. Davidson's testimony is not appropriate because it holds Defendant to "an improper standard allegedly followed in a different industry than that" in which Defendant is engaged. (Doc. No. 23 at 31.) However, Dr. Davidson will testify that product stewardship is a standard that applies generally in the chemical manufacturing industry:

> Q: Well, that's why I want to know whether or not you know whether it's Dow or whether it's anybody, what they do for the product—that's why it's so important that I want you to focus on industrial cleaning supplies and what other manufacturers do, what the industry standard does when it comes to product stewardship in this area?
>
> A: I'm not going to allow you to limit this to cleaning chemicals. Cleaning chemicals are just one of thousands of chemicals that fall under product stewardship. Product stewardship is a principle. It applies generally across the board.
>
> ....

Q: [Y]ou're citing general product stewardship philosophy, and you cited the multi-employer workplace. Is there anywhere else that you are getting the concept that Ecolab had a product stewardship duty?

A: It's, basically, coming from Dow's influence over the Manufacturing Chemists Association, which I don't even know if Ecolab is a member.... To the extent that they're a member, even if they're not a member, it's what the industry recommends and practices.

(Davidson Dep. at 204, 207.) Since the concept of "product stewardship" appears to be an accepted industry standard in the area of chemical safety, Dr. Davidson's testimony with regard to this standard is not inappropriate. *See* ⚑*Ebenhoech v. Koppers Indus. ., Inc.,* 239 F.Supp.2d 455, 467 (D.N.J.2002) (suggesting that courts generally consider the following when assessing the reliability of expert testimony under Rule 702: "whether the expert considered (1) federal design and performance standards, (2) standards established by independent standards organizations, (3) relevant literature, (4) evidence of industry practice ...") (citing ⚑*Milanowicz v. The Raymond Corp.,* 148 F.Supp.2d 525, 536 (D.N.J.2001)).

*12 It is also Dr. Davidson's opinion that the MSDS for Oasis 115XP use solution is defective. He believes the warning on the MSDS is improper because it was not explicit and strong enough in indicating the dire consequences of exposure. (Davidson Report at 5.) To support this opinion, Dr. Davidson again cites various OSHA and ANSI regulations. Defendant contends that Dr. Davidson's testimony regarding the problematic MSDS should be excluded because Defendant has no duty to provide an "MSDS for the use solution." [8] (Doc. No. 36.) Specifically, Defendant argues that Defendant has no duty to provide an MSDS for the use solution pursuant to the HCS, 29 C.F.R. § 1910.1200(d)(5)(ii), because the concentration level of the active chemicals in the solution (ammonium and potassium hydroxide) was only 0.05 percent. (Doc. No. 23 at 36.) This argument misconstrues the text of the regulation. The relevant provision states:

(5) The chemical manufacturer, importer or employer shall determine the hazards of mixtures of chemicals as follows:

...

(ii) [i]f a mixture has not been tested as a whole to determine whether the mixture is a health hazards, the mixture shall be assumed to present the same health hazards as do the components which comprise one percent (by weight or volume) or greater of the mixture

...

(iv) If the chemical manufacturer, importer, or employer has evidence to indicate that a component present in the mixture in concentrations of less than one percent ... could be released in concentrations which ... could present a health risk to employees in those concentrations, the mixture shall be assumed to present the same hazard.

29 C.F.R. § 1910.1200(d)(5)(ii), (iv). Defendant contends that because the ammonium and potassium hydroxide comprised less than one percent of the solution, they had no duty to determine the hazards of the mixture, and create an MSDS for the solution. (*Id.*) Even if we were to agree with Defendant that subsection (ii) does not create a duty on Defendant to provide an MSDS for the use solution, subsection (iv) clearly creates such a duty. It is entirely possible that through a product defect in the dispenser, as could be the case here, a higher concentration of 115XP could be added to the water creating a solution with a higher concentration of ammonium and potassium hydroxide that could present a health risk to an employee such as Plaintiff. [9] As Defendant did create an MSDS for 115XP, and Defendant had a duty to do so, Dr. Davidson's testimony regarding the problems with the MSDS is relevant and admissible. [10]

Davidson's Testimony: Dispenser Defects

Dr. Davidson criticizes the design of the solution dispenser in two respects. First, he indicates that the design is flawed because it lacked proper warnings. Second, he indicates that the design was defective because there was no automatic switch which would have allowed only "a fixed or preset amount of hot use solution into the 35 pint mop bucket." (Davidson Report at 8.) Defendant argues that it has no duty to label the chemical dispenser, so that any testimony regarding the failure to place a warning on the dispenser is not relevant. While Defendant makes the argument that it has no duty to place a warning on the dispenser, it also argues that the warning that it *did* place on the dispenser

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was in accordance with OSHA standards. Even assuming that Defendant is right that it has no duty under OSHA, the notion of product stewardship and the existence of industry standards may create such a duty. Certainly, Dr. Davidson can testify as to the industry standards related to the labeling of hazards. Moreover, Defendant's contentions regarding the labeling on all of its products are not arguments that go to the admissibility of Dr. Davidson's expert testimony. Rather, they are arguments that go to the credibility of the testimony, as judged by the ultimate trier of fact. *Kannankeril,* 128 F.3d at 809–10.

 *13 Dr. Davidson's second concern is that the dispenser lacked an automatic shutoff. He indicates that if the dispenser had an automatic shutoff feature, the mop bucket that Plaintiff was filling would not have over filled and this accident would have been avoided. Compounding the problem with this design flaw, Dr. Davidson also indicates that Defendant erred in not recommending that a bucket with a bottom drain be used in conjunction with its dispenser. [11]

In contending that Dr. Davidson's opinions regarding the design defects should be excluded, Defendant again questions Dr. Davidson's qualifications and methodology. Defendant argues that this case is similar to the case of *Chester Valley Coach Works, et al. v. Fisher Price,* No. Civ. A. 99–4197, 2001 WL 1160012, at *10 (E.D.Pa. Aug.29, 2001), where we excluded the expert's testimony because the expert "did not rely on any testing, experimentation or generally accepted texts or treatises to support [the conclusion]. Instead, his conclusion ... rests, by his own admission, solely on his experience and education." Defendant's reliance on *Chester Valley* is misplaced. The expert in *Chester Valley* was offering an opinion on the cause and origin of a fire. The expert cited the accepted methodology for reaching his conclusion, but then completely failed to follow this cited methodology, in reaching his conclusions. *Id.* at 8. In the instant case, Davidson not only relies on his knowledge and experience, but cites industry standards as well as regulations promulgated to ensure workplace safety. In *Kumho Tire Co., Ltc. v. Carmichael,* the Court stated that when evaluating the methodology behind technical expert testimony the district court should apply a "flexible" approach, 526 U.S. 137, 141, 119 S.Ct. 1167, 143 L.Ed.2d 238 (1999); *see also Yarchak v. Trek Bicycle Corp.,* 208 F.Supp.2d 470, 500 (D.N.J.2002) ("When evaluating the reliability of the reasoning and methodology employed by nonscientific or technical expert witnesses, the court's main objective

is to 'ensure that an expert, whether basing testimony on professional studies, a clearly established methodology or technique, or on his own specialized knowledge, skill or experience, employs in the courtroom the same level of intellectual rigor that characterize the practice of an expert in the relevant field." ') (quoting *Kumho Tire,* 526 U.S. at 152). Dr. Davidson's testimony is based on his extensive knowledge and experience in the area of product safety. Moreover, in concluding that "Ecolab's defectively designed Dispenser, did not comport with industry standards," Dr. Davidson cited the text GUIDELINES FOR SAFE AUTOMATION OF CHEMICAL PROCESSES (American Institute of Chemical Engineers, 1993). [12] We are satisfied that Dr. Davidson's testimony regarding the product defects in the dispenser design, satisfies the requirements of *Daubert/Kumho Tire .*

Motion for Summary Judgment
Summary judgment is appropriate "if the pleadings, depositions, answers to interrogatories, and admissions on file, together with affidavits, if any, show that there is no genuine issue as to any material fact and that the moving party is entitled to a judgment as a matter of law." Fed.R.Civ.P. 56(c). In considering a motion for summary judgment, a court must view facts and inferences in the light most favorable to the party opposing the motion. *Siegel Transfer, Inc. v. Carrier Express, Inc.,* 54 F.3d 1125, 1127 (3d Cir.1995). Defendant's Motion for Summary Judgment is predicated on the exclusion of the expert testimony of Dr. Coyer and Dr. Davidson. Since we will deny Defendant's motions to exclude this expert testimony, there remain genuine issues of material fact that must be decided by a jury. Accordingly, Defendant's Motion for Summary Judgement will be denied.

 *14 An appropriate Order follows.

ORDER

AND NOW, this _____ day of May, 2004, upon consideration of Defendant Ecolab Inc.'s Motion to Exclude the Testimony of Dr. Michael J. Coyer (Doc. No. 22), Motion to Exclude the Testimony of Dr. Burton Z. Davidson (Doc. No. 23), and Motion for Summary Judgment (Doc. No. 24), it is ORDERED that:

1. Defendant's Motion to Exclude the Testimony of Dr. Michael J. Coyer (Doc. No. 22) is Denied;

2. Defendant's Motion to Exclude the Testimony of Dr. Burton Z. Davidson (Doc. No. 23) is DENIED; and

3. Defendant's Motion for Summary Judgment, (Doc. No. 24), is DENIED.

IT IS SO ORDERED.

All Citations

Not Reported in F.Supp.2d, 2004 WL 1068805

Footnotes

1	In the Complaint, Plaintiff refers to Defendants as Ecolab, and John Does (1–10), ABC, DEF, GHI and JKL who are fictitious individuals and entities, whose identities are presently unknown. (Compl.¶ 3.) We refer to Ecolab as Defendant.
2	Diplomate status is the highest status that can be achieved as a toxicologist. (Doc. No. 22 Ex. F ("Coyer Dep.") at 24.) To achieve this status, Dr. Coyer was required to exhibit excellence in experience and written testing. (*Id.*)
3	Coyer refers to various documents used in formulating his opinions. These references are reflected by the numbers set off by parentheses in the text of the opinion.
4	The four or five minute delay between spilling the solution and the onset of pain played a significant role in Dr. Coyer formulating his opinion. It was also significant in the opinions of the medical doctors.
5	Defendant contends that this MSDS was incorrect and was revised two months later. (Doc. No. 29 at 10.)
6	The designations R–1, R–2, etc. refer to the references Dr. Davidson used in preparing his report.
7	Dr. Davidson cites his tenth reference which is "various warnings standard and codes (e.g. ANSI Z129.1[,] ANSI Z535.4, and ANSI Z4000.1; and 29[C]FR 1910.120, 29 C[F]R 1910.133, 29 CFR 1900.145, and 16 CFR Parts 1500 to 1512.[)]" (Davidson Report at 2.)
8	Defendant's argument concerning the MSDS for the solution is curious since Defendant did in fact create an MSDS for the solution. (Doc. No. 23 at 38.)
9	Dr. Davidson suggests in his Report that a dispenser may be miscalibrated and therefore deliver a ratio of concentrate to water that was too high. (Davidson Report at 8.) There is no dispute that as the concentration levels of 115XP increase, the potential harm the solution could cause also increases.
10	Throughout its Motion, Defendant argues that it is not the "manufacturer" of the use solution because it was Plaintiff's employer rather than Defendant that mixed 115XP with water to create the solution. (Doc. No. 23 at 37.) Defendant manufactured 115XP, manufactured the dispenser from which 115XP was mixed with water, and installed the dispenser and the chemicals at Plaintiff's employer's business. Defendant's argument appears to be inconsistent with the intent of the HCS.
11	In the catalog cited by Dr. Davidson, there are two types of mop buckets made by Rubbermaid: one with a bottom drain plug with splashguard, and one without. If Defendant recommended the bucket with bottom drain plug this accident could also have been averted.
12	In his report, Dr. Davidson also generally cites PRODUCT SAFETY – SAFETY BY DESIGN: REDUCING HAZARDS THROUGH BETTER DESIGNS 29–33 (Professional Safety, Vol. 43, No.2), and STONE AND

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WEBSTER ENGINEERING CORPORATION, RISK ASSESSMENT AND RISK MANAGEMENT FOR THE CHEMICAL PROCESS INDUSTRY (1991).

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Exhibit 38

Table III. Mass Spectra[a] of Cyclic Silanes

Ion	$(CH_2)_4$-SiH_2[b]	$(CH_2)_3$-SiH_2[d]
M + 1	...	1.3
M	9.4	12.3
M − 1	3.0	7.1
M − 2	1.5	8.1
M − CH_2 + 1	...	2.1
M − CH_3	0.9	4.3
M − CH_2 − 2	...	1.4[e]
M − CH_2 − 3	...	4.1[e]
M − CH_2 − 5	...	2.6[e]
M − $2CH_2$ + 1	...	7.0
M − 28	48.3	13.6
M − 29	4.8	10.8
M − 30	~3[c]	6.9
M − 31	4.4	3.3
M − 33	~2[c]	4.0[e]
SiH_3^+	...	5.1
SiH_2^+	...	0.6
SiH^+	4.5	4.3
Si^+	3.1	} 14.5
M − CH_2SiH_2	1.4	

[a] Spectra at 70-v ionizing voltage; intensities given as per cent of total intensity above m/e 40. [b] Reference 12. [c] Estimated from given spectra. [d] Corrected to spectra expected for ^{28}Si, ^{12}C, ^1H. [e] Peaks that have essentially vanished in spectra at 8.2-v ionization potential.

negative shift of the α protons in $(CH_2)_3SiH_2$. This effect is reduced when the silicon hydrogens are replaced by halogens; in this sense, the $SiCl_2$ group appears less electropositive than SiF_2.

Mass Spectra. Mass spectra of both $(CH_2)_3SiH_2$ and $(CH_2)_3SiD_2$ were obtained. The important ions observed at an ionizing potential of 70 v for the nondeuterated species are compared in Table III to those found for $(CH_2)_4SiH_2$ as reported by Duffield and co-workers.[12] Spectra were also obtained at lower voltages. The percentage contribution of the molecular ion to the spectrum above m/e of 40 increased from 12.3% at 70 v to 20.9 (15), 29.5 (10), and 36.0% (8.2 v). In the spectrum at 70 v, over one-half of all intensity is distributed between 31 and 25 m/e; at 8.2 v this is only about 10%.

In general, the spectrum of the four-membered ring silane is richer in the number of peaks observed than that of silacyclopentane. However, some of these additional ions in the silacyclobutane spectrum were not observed at lower ionizing potentials. Both spectra have strong molecular ion peaks; both have a large intensity at M − 28, implying a loss of ethylene. Both spectra have M − 1 and M − 2 ions resulting primarily from Si–H bond cleavage. The 1,1-d_2 analogs of each give spectra with peaks corresponding to loss of one or two deuterium atoms.

A significant difference in the spectra of the two silanes is that in silacyclobutane peaks were observed at M − nCH_2 + 1 (n = 1–3). Thus, in the four-membered ring, Si–C bond cleavage seems to be accompanied by hydrogen transfer; this process appears unimportant in silacyclopentane fragmentation.

(12) A. M. Duffield, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **87**, 2920 (1965).

Nitrosative Cleavage of Tertiary Amines[1]

Peter A. S. Smith and Richard N. Loeppky

Contribution from the Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48104. Received September 6, 1966

Abstract: Tertiary amines react with aqueous nitrous acid, contrary to common belief, and undergo dealkylation to form a carbonyl compound, a secondary nitrosamine, and nitrous oxide. The ratios of products from *meta*- or *para*-monosubstituted tribenzylamines are affected but little by the electronic influence of the substituents, and obey the Hammett equation with a small negative value of the reaction constant. Susceptibility of an amine to nitrosative cleavage is markedly reduced by base-weakening effects, and is prevented altogether by quaternization. Quinuclidine, in which the tertiary nitrogen is at a bridgehead, is inert. α substituents in tribenzylamines and benzyldiethylamines strongly shift dealkylation to the unsubstituted groups, regardless of the electronic character of the α substituent (*e.g.*, alkyl or carboethoxy). Tribenzylamine-α,α-d_2 shows a deuterium isotope effect k_H/k_D = 3.78. The facts are correlated by a mechanism (eq 4–6) involving formation of an N-nitrosoammonium ion and *cis* elimination of nitroxyl to form a ternary immonium ion, R_2N^+=CR_2, which then undergoes hydrolysis and further nitrosation. Nitrosyl fluoroborate and tribenzylamine form an unstable 1:1 addition compound at −45°; above −20°, this substance decomposes to form tribenzylammonium and N,N-dibenzylbenzaldimmonium fluoroborates.

The belief that tertiary amines do not react with aqueous nitrous acid is probably the most persistent myth in organic chemistry, notwithstanding a veritable parade of experimental refutations extending over an even century. Scarcely a textbook currently in print suggests that aught beyond salt formation occurs, and the assumed inertness of tertiary amines has been made the basis of a test for distinguishing them from primary and secondary amines.
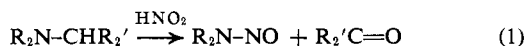
In actual fact, Guether[2] reported correctly in 1864

(1) Presented at the XIXth I.U.P.A.C. Congress, London, 1963; from the doctoral dissertation of R. N. L.

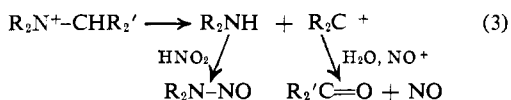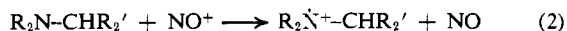(2) B. Guether, *Arch. Pharm.*, [2] **123**, 200 (1864).

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that triethylamine is converted to diethylnitrosamine by aqueous nitrous acid. Unfortunately, he was taken to task 2 years later by Heintz[3] for allegedly having used impure amine, and Heintz went on to claim that highly purified triethylamine remained unattacked. However, Heintz used very different conditions from Guether's, and apparently paid no attention to the possibility that the occurrence of further reaction might be dependent on time, temperature, and acidity. Heintz's paper is apparently the origin of the widely accepted test mentioned above.

The history of the many independent rediscoveries of the cleavage of tertiary amines by nitrous acid has recently been reviewed by Hein,[4] and need not be repeated here. The most recent encounter with the phenomenon occurred in this laboratory, as an outgrowth of nitrosation experiments with substituted hydrazides.[5]
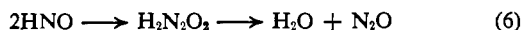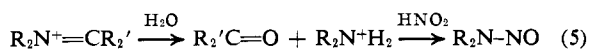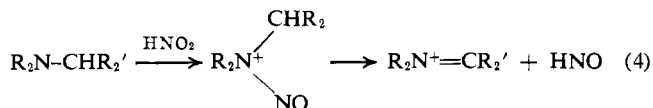
The accumulated reports of the cleavage of tertiary amines with nitrous acid lead to a clear generalization that an alkyl group is removed oxidatively, and appears as an aldehyde or ketone, the nitrogenous portion being converted to a nitrosamine (eq 1); further details of the stoichiometry are obscure. Three quite different mechanisms have been proposed. According to one view,[6] a free-radical process may be involved, in which the amine is first oxidized to an aminium ion radical, which then fragments to secondary amine and a methylene cation radical, from which a carbonyl compound is generated by reaction with water and further oxidation by nitrosonium ion (eq 2 and 3). According
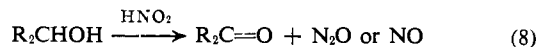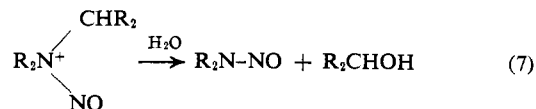
$$R_2N\text{-}CHR_2' \xrightarrow{HNO_2} R_2N\text{-}NO + R_2'C\text{=}O \qquad (1)$$

$$R_2N\text{-}CHR_2' + NO^+ \longrightarrow R_2\dot{N}^+\text{-}CHR_2' + NO \qquad (2)$$

$$R_2N^+\text{-}CHR_2' \longrightarrow R_2NH + R_2C^+ \qquad (3)$$

to another view,[5] the mechanism is probably heterolytic, involving elimination of nitroxyl from a nitrosammonium ion to form an immonium ion, which is then hydrolyzed (eq 4, 5, and 6).

$$R_2N\text{-}CHR_2' \xrightarrow{HNO_2} R_2N^+ \begin{smallmatrix} CHR_2 \\ \diagup \\ \diagdown \\ NO \end{smallmatrix} \longrightarrow R_2N^+\text{=}CR_2' + HNO \quad (4)$$

$$R_2N^+\text{=}CR_2' \xrightarrow{H_2O} R_2'C\text{=}O + R_2N^+H_2 \xrightarrow{HNO_2} R_2N\text{-}NO \quad (5)$$

$$2HNO \longrightarrow H_2N_2O_2 \longrightarrow H_2O + N_2O \qquad (6)$$

The third mechanism[7] involves nucleophilic dealkylation to give a secondary nitrosamine and an alcohol (or alkyl halide), analogous to the cleavage of tertiary amines with phosgene or cyanogen bromide.[8] The carbonyl compounds obtained are supposed to result from subsequent oxidation. This mechanism was originally formulated somewhat indefinitely as a four-center process, but can more reasonably be imagined

(3) W. Heintz, *Ann.*, **138**, 319 (1866).
(4) G. Hein, *J. Chem. Educ.*, **40**, 181 (1963).
(5) P. A. S. Smith and H. G. Pars, *J. Org. Chem.*, **24**, 1324 (1959).
(6) I. Glazer, E. D. Hughes, C. K. Ingold, A. T. James, G. T. James, and E. Roberts, *J. Chem. Soc.*, 2671 (1950).
(7) R. Wegler and W. Frank, *Ber.*, **69**, 2071 (1936); **70**, 1279 (1937).
(8) J. von Braun, *ibid.*, **33**, 1438 (1900).

as nucleophilic displacement on an α carbon of an N-nitrosoammonium ion (eq 7 and 8). It can be

$$R_2N^+ \begin{smallmatrix} CHR_2 \\ \diagup \\ \diagdown \\ NO \end{smallmatrix} \xrightarrow{H_2O} R_2N\text{-}NO + R_2CHOH \qquad (7)$$

$$R_2CHOH \xrightarrow{HNO_2} R_2C\text{=}O + N_2O \text{ or } NO \qquad (8)$$

seen at once that the stoichiometry demanded by the respective mechanisms is different, and that the product-determining steps are of different kind. The present study of the reaction consists of investigations of the stoichiometry, structural requirements, and product selectivity, in the light of the foregoing and other reasonable mechanisms.

Results

Tribenzylamine and N,N-dibenzylaniline and their substituted derivatives were chosen for study because they are among the few simple tertiary amines that are solids at ordinary temperatures, at the same time allowing systematic variation of substituent influences. These amines were prepared in the customary way by treatment of primary or secondary amines with benzyl halides or by reductive alkylation.

It had already been shown that the cleavage of tertiary amines with nitrous acid is slow at 0° and is retarded in strongly acid medium, for which reasons warm, aqueous acetic acid was chosen as the medium in these experiments.[5] It did not seem practical to determine the stoichiometry of nitrous acid consumption directly under such conditions, owing to the instability of nitrous acid and the loss of oxides of nitrogen. One experiment was nevertheless conducted with equimolar amounts of nitrous acid (from sodium nitrite) and tribenzylamine. Unreacted amine, which could be recovered fairly efficiently, amounted to 50%, and the purified dibenzylnitrosamine obtained amounted to 38% of the original amine. The discrepancy in the material balance, 12%, is believed to be largely if not entirely a result of mechanical losses on separation and purification. Optimum yields of purified cleavage products, amounting generally to about 80% of benzaldehydes and 60% of nitrosamines, were obtained only with a large excess of nitrous acid, about 10 molar equiv.

More significant information about the stoichiometry was obtained from an experiment in which the evolved gases were collected. Nitric oxide and nitrogen dioxide, inevitably formed from the decomposition of nitrous acid, were removed by scrubbing with strongly alkaline potassium permanganate solution. There remained a volume of gas equivalent to nearly 0.5 mole per mole of amine used; mass spectrometric examination showed it to consist largely of nitrous oxide, with minor contamination by air and nitric oxide.

Some N,N-dibenzylanilines were examined in order to obtain information about the importance of the basicity of the amine. N,N-Dibenzylaniline itself could not, of course, be examined for nitrosative cleavage, since nitrosation of the aniline ring would take place rapidly; the p-chloro and p-nitro derivatives, which are free of this objection, were therefore used. N,N-dibenzyl-p-chloroaniline reacted smoothly under the same conditions as tribenzylamine; benzaldehyde

(42%) and benzyl-*p*-chlorophenylnitrosamine (62%) were obtained normally, although in slightly reduced yield. Under the same conditions, N,N-dibenzyl-*p*-nitroaniline was recovered unchanged in 99% yield. N,N-Dibenzyl-*p*-anisidine and N,N-dibenzyl-*p*-toluidine were also examined; both underwent cleavage, as shown by the formation of appreciable amounts of benzaldehyde, but other reactions predominated. No tertiary amine was recovered in either case.[9] Although it was thus not possible to attain the original objective of comparing the susceptibility to nitrosative dealkylation under controlled conditions as a function of substituent effects on the aniline ring, it is evident from the inertness of the *p*-nitro member, which must be by far the weakest base, that a qualitative parallel exists between susceptibility and base strength.

To determine whether the unshared electron pair of the tertiary amine free base is necessary for nitrosative dealkylation, the quaternary derivative, methyltribenzylammonium nitrate was subjected to the usual nitrosating treatment at 95° for 45 min. The original compound was recovered in 70% yield, and no aldehyde or nitrosamine could be detected. A similar experiment with tribenzylamine N-oxide, however, did result in cleavage to benzaldehyde and dibenzylnitrosamine; these are the products obtained from tribenzylamine itself however.

Quinuclidine was subjected to the same reaction conditions to determine whether resistance to double-bond formation at the bridgehead nitrogen would interfere with dealkylation. The starting material was recovered in 92% yield.

The effect of essentially electronic factors was investigated by means of a group of tribenzylamines each bearing a *para* substituent on one benzyl group. In an orienting series of experiments, the mixed aldehydes and the mixed nitrosamines were isolated by distillation. The composition of the aldehyde mixture was then determined by fractional distillation and by vapor phase chromatography; the nitrosamines were converted to secondary amines by treatment with urea and alcoholic hydrochloric acid, and then fractionated. The results are collected in Table I. The effect of temperature was examined by experiments covering a 30° range. For these experiments, the *p*-nitro derivative was not used, owing to its incomplete reaction. Only the aldehydes were used to determine product ratios in this group of experiments, since they were obtained in higher yields than the amines. The compositions were determined by vpc, standardized against known mixtures; the results are collected in Table II.

The effect of α substitution on the nitrosative cleavage of tribenzylamine was investigated with the α-methyl, α-ethyl, and α-carboethoxy derivatives. For comparison, benzyldiethylamine and its α-methylbenzyl and α-carboethoxybenzyl analogs were also studied, along with dibenzylhexahydrobenzylamine. The product ratios were determined either by distillation (with or without hydrolysis of the nitrosamines) or by vpc, as feasibility and reliability indicated. Inasmuch as the reactions were in some cases far from complete, and in others were accompanied by secondary

Table I. Product Ratios from the Nitrosative Cleavage of Monosubstituted Tribenzylamines, $RC_6H_4CH_2N(CH_2C_6H_5)_2$, at 90–95°

R	Total aldehydes, %	Mole ratio, C_6H_5CHO/RC_6H_4CHO	Total secondary amines, %	Mole ratio, $C_6H_5CH_2NHCH_2C_6H_4R/(C_6H_5CH_2)_2NH$
p-CH₃O	82	1.468[a] 1.758[b]	69	1.95
m-CH₃	86	2.12[a] 1.95[b]	59	1.95
p-Cl	81	2.12[a]	61	2.1
p-NO₂	39[c]	2.64[a]	53[c]	2.5

[a] By fractional distillation. [b] By vpc. [c] Not corrected for an appreciable amount of unreacted starting material.

Table II. Effect of Temperature on Product Ratios from Monosubstituted Tribenzylamines, $RC_6H_4CH_2N(CH_2C_6H_5)_2$

R	C_6H_5CHO/RC_6H_4CHO		
	62.8°	81.3°	93.8°
p-CH₃O	1.35	1.61	1.88
m-CH₃	1.71	2.00	1.94
p-Cl	2.11	2.22	1.88

reactions, and some of the products underwent partial decomposition during distillation or chromatography, the ratios determined are more qualitative than quantitative; however we believe the yields of products isolated to provide reliable minima. The results are collected in Table III.

Tribenzylamine-α,α-d_2 was examined for the appearance of an isotope effect in nitrosative cleavage. The required substance was prepared by reduction of N,N-dibenzylbenzamide with lithium aluminum deuteride; the percentage of deuteration determined by the falling-drop method of Keston,[10a] was 88.5%. The benzaldehyde obtained by nitrosative cleavage at 95° was examined for deuterium content by the infrared method of Wiberg;[10b] it contained 7.8% of deuteriobenzaldehyde. The dibenzylnitrosamine was examined by means of its integrated nmr spectrum; about 7% of it was deuterium free. The isotope effect, k_H/k_D, was calculated from these data using a statistical effect of 1:3; the value obtained from the benzaldehyde analysis, 0.333 × 88.5/7.8 = 3.78, is considered to be the more reliable.

Brief attention was also given to the closely related reactions of nitrosyl chloride and nitrosyl fluoroborate with tertiary amines in anhydroxylic medium. Jones and Whalen[11] have reported that nitrosyl chloride and trimethylamine form an addition compound at low temperatures, and that it decomposes below 0° to give off nitric oxide and leave a colorless solid, from which trimethylamine and dimethylamine salts could be obtained. We obtained analogous results with tribenzylamine, which gave as a major product tribenzylamine hydrochloride, together with substantial quantities of another salt partially separable from the former by solution in benzene. The more soluble salt was readily

(9) Other examples of competition of ring substitution with nitrosative cleavage of dialkylanilines have been reported by G. P. Crowley, G. J. G. Milton, T. H. Reade, and W. M. Todd, *J. Chem. Soc.*, 1286 (1940).

(10) (a) A. S. Keston, D. Rittenberg, and R. Schoenheimer, *J. Biol. Chem.*, 122, 227 (1938); (b) K. Wiberg, *J. Am. Chem. Soc.*, 76, 5371 (1954).
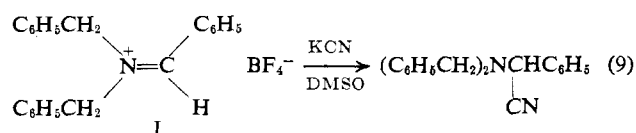(11) L. W. Jones and H. F. Whalen, *ibid.*, 47, 1343 (1925).

1150

Table III. Nitrosative Cleavage of Various Tertiary Amines $R_2CHN(CHR_2')_2$

R_2CH	$R_2'CH$	Products isolated, %[a]	Cleavage ratio,[b] $R_2CH/R_2'CH$
$C_6H_5CH(COOEt)$	$C_6H_5CH_2$	C_6H_5CHO 50	<1/99[c]
$C_6H_5CH(Et)$	$C_6H_5CH_2$	C_6H_5CHO, 80; C_6H_5COEt, 1.7; $R_2CHNHCHR_2'$, 82	2/98
$C_6H_5CH(CH_3)$	$C_6H_5CH_2$	$(C_6H_5CHO, C_6H_5COCH_3)$	8/92[d]
$(CH_2)_5CHCH_2$	$C_6H_5CH_2$	C_6H_5CHO, 50; $(CH_2)_5$-$CHNHCHC_6H_5$	<1/100
$C_6H_5CH(COOEt)$	Et	R_2CO, 16.4; $R_2CHN(Et)NO$, 57	22/78
$C_6H_5CH(CH_3)$	Et	R_2CO, 3; $R_2CHN(Et)NO$, 38	8/92
$C_6H_5CH_2$	Et	C_6H_5CHO, 16; $C_6H_5CH_2N(Et)NO$, 57.5	21/79

[a] Other products, particularly acetaldehyde and diethylnitrosamine, were occasionally isolated, but are not reported here because the yields were not felt to be sufficiently reliable. [b] Normalized to a basis of 100. [c] Vpc analysis showed only benzaldehyde. [d] Vpc analysis.

hydrolyzed by hot water to give dibenzylamine hydrochloride and benzaldehyde, but unfortunately could not be obtained pure. Further experiments were carried out using nitrosyl fluoroborate instead, in the unrequited hope of obtaining a more readily purified product.

Nitrosyl fluoroborate suspended in chloroform reacted with tribenzylamine at $-10°$ to give chloroform-soluble products, which were precipitated as a mixture of colorless solids by addition of petroleum ether. Hydrolysis with hot water produced tribenzylamine and dibenzylamine hydrochlorides and benzaldehyde. The infrared spectrum of the solid mixture was distinguished by absorptions at 3200 and 1650 cm^{-1}, and the absence of absorption at 3400 cm^{-1}, where dibenzylamine fluoroborate absorbs. On standing in moist air, the solid slowly lost the 1650 band, and one at 3400 cm^{-1} developed. These features agree with the presence of tribenzylammonium fluoroborate (infrared at 3200 cm^{-1}) and N,N-dibenzylbenzaldimmonium fluoroborate (I) (infrared at 1650 cm^{-1} attributable to C=N$^+$); moisture would convert the latter compound to dibenzylammonium fluoroborate (cf. eq 5), thus accounting for the changes in the spectrum. The nmr spectrum of a chloroform solution of the solid mixture confirmed these deductions. In addition to the aromatic proton signals, there was a singlet at τ 0.50, attributable to the aldimmonium hydrogen, a doublet at τ 5.6, and two equal singlets, at τ 4.78 and 4.61. The doublet, whose area showed no whole-number relationship to any of the singlets, is attributed to tribenzylammonium fluoroborate, which gives an identical signal. The two singlets can be attributed to the *cis-* and *trans*-benzyl group methylenes of the immonium salt. The relative areas of the two singlets and the doublet indicated about 40% of immonium salt in the mixture. The identity of I in the product was further confirmed by hydrolysis, which gave benzaldehyde (18%) and a mixture of dibenzylamine and tribenzylamine in a ratio of 1:1.8. Furthermore, treating the mixture with potassium cyanide in dimethyl sulfoxide gave α-cyanotribenzylamine in good yield (eq 9).

$$\begin{array}{c} C_6H_5CH_2 \quad\quad C_6H_5 \\ \overset{+}{N}=C \quad BF_4^- \xrightarrow[DMSO]{KCN} (C_6H_5CH_2)_2NCHC_6H_5 \quad (9) \\ C_6H_5CH_2 \quad\quad H \\ \quad I \quad\quad\quad\quad\quad CN \end{array}$$

The reaction of nitrosyl fluoroborate and tribenzylamine gave rise to a different initial product when car-

ried out at $-55°$; a red substance, insoluble in chloroform below about $-20°$, appeared. At temperatures of $-20°$ or above, the red substance slowly lost its color and evolved gas; examination of the residue showed it to be a mixture of salts similar to what had been obtained directly at higher temperatures. Treatment of the red precipitate with alcoholic potassium hydroxide at $-45°$ produced tribenzylamine and potassium nitrite, in an approximately 1:1 ratio, and no dibenzylamine, which shows that formation of immonium salt had not yet taken place. The red compound is thus presumably N-nitrosotribenzylammonium fluoroborate (II). The possibility that it

$$(C_6H_5CH_2)_3N^+\text{-}NOBF_4^- \xrightarrow[-45°]{KOH} (C_6H_5CH_2)_3N + KNO_2 \quad (10)$$
$$\quad\quad\quad II$$
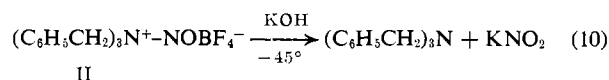
might be merely a mixture of the reactants is rendered unlikely by the fact that tribenzylamine is soluble in chloroform under the conditions of the experiments.

Attempts to reduce II to a quaternary hydrazinium salt with lithium aluminum hydride invariable brought about N–N cleavage, even at low temperatures, and only tribenzylamine was obtained. Reduction was also explored using the N-methylpiperidine–nitrosyl fluoroborate adduct, but the only reduction product that could be obtained was N-methylpiperidine–borane (eq 11). Treatment of the corresponding quaternary

$$(11)$$
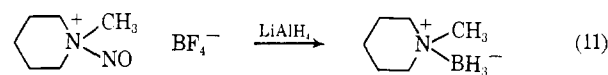
hydrazinium salt, N-methyl-N,N-pentamethylenehydrazinium fluoroborate, with lithium aluminum hydride gave the same product.

Discussion

In addition to the previously proposed mechanisms cited at the beginning of this paper, one more deserves consideration. It is bimolecular hydride abstraction from an α carbon by nitrosonium ion, giving nitroxyl and the same immonium species that appears in eq 4 and 5. The stoichiometry that we have determined, and particularly the formation of nitrous oxide, eliminates only the cation radical mechanism (eq 2 and 3). The implications of the remaining evidence will therefore be discussed in terms of the three other mechanisms.

The over-all implication of the various reports of nitrosative cleavage over the past century is that a rela-

tively weakly acid solution is required. In fact, the failure of Heintz[3] to observe any cleavage whatsoever may be attributed to the fact that he used concentrated hydrochloric acid as his medium, in contrast to Guether,[2] whose work he purported to repeat. Furthermore, the reaction generally requires mild warming for best results, and thus may be regarded as intrinsically slower than the reaction of primary and secondary amines with nitrous acid (however, even some primary amines require heating for reaction). It is this difference in reactivity, although it is not sharp, together with the customary use of fairly strong hydrochloric acid, that is presumably responsible for such reliability as there is in the use of nitrous acid as a test to distinguish among the classes of amines.

It appears that the function of acid in nitrosative cleavage is to generate the required nitrosating species,[12] but that reaction is retarded or prevented insofar as the amine is at the same time converted to its conjugate acid. This view is strongly supported by the fact that the quaternary compound, methyltribenzylammonium nitrate, did not undergo cleavage. A related experiment directed to the same point, the exposure of tribenzylamine oxide to nitrosative cleavage conditions, was not of value, for it was found that nitrous acid also achieves reduction (presumably before C–N cleavage), and gives the same products as obtained from the tertiary amine. The apparent necessity for an unshared electron pair weighs against the bimolecular hydride abstraction mechanism, but it is not proof, inasmuch as the positive charge on an ammonium ion would assuredly hinder the availability of hydride to an approaching cation such as nitrosonium ion. The experiments with base-weakening substituents on dibenzylanilines however, particularly the inertness of N,N-dibenzyl-p-nitroaniline, are more forceful. The electronic effects of the nitro group should be relatively small on the benzylic hydrogens, whereas its effect on the availability of the unshared electron pair on nitrogen is large, as revealed by the base strength of aniline (pK_a = 4.59) compared to that of p-nitroaniline (pK_a = 1). The formation of the N-nitrosoammonium ion, a feature of both the elimination mechanism and the dealkylation mechanism, would be strongly retarded by such base-weakening substitution.

The inertness of quinuclidine, which is a respectably strong base (pK_a = 10.58) may be taken as evidence that a C=N double bond, which could not form readily to a bridgehead atom, may be involved in nitrosative cleavage. The nucleophilic dealkylation path, however, would also be denied by the cage structure, so that this experiment really casts doubt only on the cation radical mechanism.

The effects of ring substitution on product selection in tribenzylamines (Tables I and II) show that susceptibility to electronic effects is low, and that electron withdrawal retards removal of the group involved. The results can be correlated by the Hammett equation in the form log (ArCHO/0.5C$_6$H$_5$CHO) = $\rho\sigma$, for the results are presumably determined by the relative rates of cleavage involving the respective groups. The values of ρ are -0.39 at $62.8°$, -0.25 at $81.3°$, -0.14 at $93.8°$, and -0.17 for the pilot experiments at 90–$95°$. A plot of these values against the reciprocal temperature gives

a straight line obeying the equation $\rho = -0.08/T + 2$; an isokinetic temperature of $117°$ is indicated. It is probably unwise to read deep meaning into these figures, in view of the limitations of the data and the proximity to the isokinetic temperature, but it is safe to say that the results can be reconciled qualitatively with all three remaining mechanisms. The negative value of ρ is consistent both with dealkylation by an SN1 process (involving formation of benzyl cations) and with removal of a benzylic hydrogen with its electron pair (bimolecular or intramolecular). The pronounced deuterium isotope effect, however, virtually eliminates nucleophilic dealkylation mechanisms (SN1 or SN2), for product selection would be subject only to a secondary, not a primary, deuterium isotope effect. Primary isotope effects of slightly lower magnitude (1.8 to 2.2) have, indeed, been observed[13] in the pyrolysis of xanthate and acetate esters to form olefins, reactions that are well established as examples of *cis* elimination.

It should be noted at this point that the elimination mechanism may be intramolecular, involving a cyclic transition state, which implies a *cis* geometry for the process, or it may be bimolecular, involving an external base (or simultaneous proton acceptor–donor) to

$$(12)$$
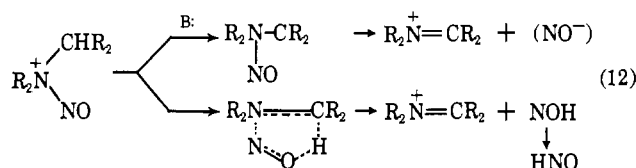
remove a proton from an α carbon (eq 12). The negative value of the Hammett reaction constant with substituted tribenzylamines is good evidence against bimolecular base-catalyzed elimination, which requires a protonic α hydrogen, and therefore implies the intramolecular process and the geometrical consequences of *cis* elimination. The experiments with α-substituted tertiary amines (Table III) are directed to this point. Even though the significance of the results is limited by the lack of good material balance, the essential point is inescapable, that the electronic nature of the α substituent is of minor significance compared with its steric effect. For example, the overwhelming formation of benzaldehyde from both α-ethyl- and α-carboethoxy-tribenzylamine is not reconcilable with the opposite electronic influences of these two substituents.

All of the observations of the effects of α substituents can be correlated on the basis of nonbonded interactions in a cyclic transition state. This may be done by means of Newman projections (III and IV). Rotation of an unsubstituted benzyl group into position for *cis* elimination of a hydrogen and the nitroso group brings about only one site of eclipsing not involving hydrogen; rotation of an α-substituted benzyl group into the position for elimination, on the other hand, sets up two sites where eclipsing can be expected to be accompanied by steric interference. It is thus implied that the unsubstituted benzyl groups will be preferentially cleavaged from α-substituted tribenzylamines, in agreement with all the examples investigated. By precisely analogous reasoning, it can be predicted that (apart from statistical effects) hexahydrobenzyldibenzylamine will undergo preferential cleavage of a benzyl group,

(12) T. A. Turney and G. A. Wright, *Chem. Rev.*, **59**, 497 (1959).

(13) C. H. Depuy and R. W. King, *ibid.*, **60**, 431 (1960).

whereas benzyldiethylamine will undergo preferential cleavage of an ethyl group, notwithstanding the fact that the situations are electronically similar, in each case an alkyl group being pitted against benzyl. It is only necessary to make the reasonable assumption that the steric requirements of a benzyl group are less than those of hexahydrobenzyl, but larger than those of ethyl. The results reported in Table III are in accord with these deductions.
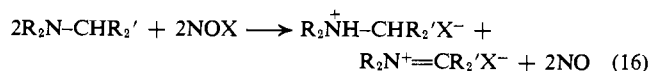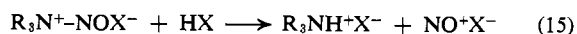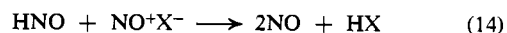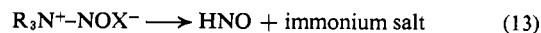
We conclude, therefore, that the process represented by eq 4–6, with *cis* elimination of nitroxyl in the product-determining step, best fits the available facts about nitrosative cleavage of tertiary amines. It should be noted, however, that N-methyl-N-ethyl-*p*-nitroaniline has been reported[9] to undergo nitrosative cleavage of the ethyl group preferentially, which is the opposite of the expectation on the basis of the foregoing considerations. On the other hand, this result was obtained by means of

III   IV
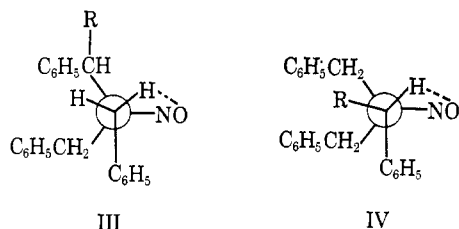
differential melting point determination of the mixed neutral products of the reaction; since no evidence was presented that the mixtures consisted wholly of the assumed pair of nitrosamines, the analysis may have been in error.

The experiments with nitrosyl chloride and fluoroborate show that the products of the reaction of these nitrosating reagents with tertiary amines are different from those of aqueous nitrous acid. The reactions may, nevertheless, be considered analogous, for the differences are precisely those that would be anticipated to result from the differences in the reaction media. In a nonhydrolytic medium, as used with the nitrosyl compounds, the reaction cannot go beyond the elimination stage (eq 4 and 12), and the immonium salt intermediate deduced for the aqueous nitrosation reactions can actually be isolated. The unstable addition compounds obtained with the nitrosyl compounds at low temperatures have the characteristics to be expected of the postulated N-nitrosotrialkylammonium salts, and their saponification to tertiary amine and nitrite in particular lends strong support to such a formulation.

The concomitant formation of tertiary amine salt, together with nitric oxide instead of nitrous oxide, when nitrosyl compounds are used as nitrosating agents, may be accounted for by reaction of the nitrosyl compounds with nitroxyl (eq 13 and 14); only a very small amount of free nitrosyl compound need remain with the nitrosammonium salt (as a result of either mechanical retention or dissociation of the salt) to provide such a path, for it would be regenerated by eq 15. The resulting stoichiometry, by which tertiary amine salt and immonium salt are produced in equimolar quantities (eq 16) would be consistent with the observed presence of an excess of tribenzylamine in the products if it can be assumed that decomposition of the nitrosammonium intermediate was not complete in the experiments with nitrosyl fluoroborate. Equation 16 is also consistent with the proportion of nitric oxide

$$R_3N^+-NOX^- \longrightarrow HNO + \text{immonium salt} \quad (13)$$

$$HNO + NO^+X^- \longrightarrow 2NO + HX \quad (14)$$

$$R_3N^+-NOX^- + HX \longrightarrow R_3NH^+X^- + NO^+X^- \quad (15)$$

$$2R_2N-CHR_2' + 2NOX \longrightarrow R_2\overset{+}{N}H-CHR_2'X^- +$$
$$R_2N^+=CR_2'X^- + 2NO \quad (16)$$

obtained by Jones and Whalen[11] from the reaction of nitrosyl chloride with trimethylamine.

Experimental Section[14]

Secondary Amines. The general preparative method involved the dropwise addition of 0.2 mole of aldehyde over a 1.5-hr period to a cold, stirred solution of 0.2 mole of the appropriate primary amine in 50 ml of absolute ethanol. This solution was then added to 0.2 g of prereduced platinum oxide and hydrogenated at ∼3 atm. Dibenzylamine[15] and its *p*-methoxy[16] and *p*-chloro[17] derivatives, N-(cyclohexanemethyl)benzylamine,[18] N-benzyl-*p*-chloroaniline,[19] N-benzyl-*p*-anisidine,[20] and N-benzyl-*p*-toluidine[21] are known compounds. *m*-Methyldibenzylamine, bp 118° (0.2 mm), was obtained as a colorless oil in 88% yield.

Anal. Calcd for $C_{15}H_{17}N$: C, 85.38; H, 8.12; N, 6.64. Found: C, 85.50; H, 8.20; N, 6.76.

N-Benzyl-1-phenylpropylamine. A solution of 58.5 g (0.3 mole) of benzylidenebenzylamine[22] in 250 ml of dry ether was added dropwise to a stirred solution of ethylmagnesium bromide which had been prepared from 14.4 g (0.6 g-atom) of magnesium and 66 g (0.6 mole) of ethyl bromide. A mildly exothermic reaction ensued, and the mixture was heated under reflux overnight. After treatment of the cooled mixture with a saturated solution of ammonium chloride, filtration from salts, and drying over potassium carbonate, the ether was removed and the amine was distilled at 118–120° (0.75 mm) to give 27 g (0.12 mole, 40%) of pure N-benzyl-1-phenylpropylamine. (The hydrochloride melted at 171°.)

Anal. Calcd for $C_{16}H_{18}N$: C, 85.40; H, 8.45; N, 6.17. Found: C, 85.59, H, 8.53; N, 6.17.

Tertiary Amines. Most of the tertiary amines were prepared by procedures analogous to the preparation of 4-methoxytribenzylamine, described here. Benzyl bromide (30 g, 0.15 mole) was added to a stirred mixture of 34 g (0.15 mole) of 4-methoxydibenzylamine, 45 ml of glycerol, and 16 g of sodium carbonate. The flask was flushed with nitrogen, and the mixture was heated with stirring at 150° for 24 hr. The cooled reaction mixture was poured into 250 ml of water and extracted with three 100-ml portions of ether. The ether was removed from the combined, dried extracts, and the residual oil was distilled to give 40 g (0.126 mole, 84%) of 4-methoxytribenzylamine, bp 190° (0.4 mm). The results for the other tertiary amines are collected in Table IV.

N,N-Dibenzyl-4-chloroaniline. A mixture of 127 g (1 mole) of 4-chloroaniline, 125 g of sodium carbonate, 150 ml of glycerol, and 253 g (2 moles) of benzyl chloride was heated at 150° with stirring under nitrogen for 18 hr, before being cooled and poured into 500 ml of water. The aqueous mixture was extracted with three 350-ml portions of ether, and the ether solution was dried over anhydrous sodium sulfate, filtered, and stripped of ether to give an oil, which solidified to a white solid. Recrystallization from ethanol gave 202 g (66%) of N,N-dibenzyl-4-chloroaniline, mp 101–103°.

Anal. Calcd for $C_{20}H_{18}ClN$: C, 77.76; H, 5.90; N, 4.41. Found: C, 77.92; H, 6.01; N, 4.50.

N,N-Dibenzyl-4-methoxy-2-nitroaniline, prepared similarly from benzyl bromide and 4-methoxy-2-nitroaniline,[23] was obtained as

(14) Infrared spectra were determined on a Perkin-Elmer Infracord. Nmr spectra were determined with a Varian Associates A-60 instrument, and are referred internally to tetramethylsilane. Microanalyses are by Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points are uncorrected.

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Table IV. Tertiary Amines

Product, tertiary amine	Alkyl halide	Source, secondary amine	Mp or bp (mm), °C	Calcd, %			Found, %			Yield, %
				C	H	N	C	H	N	
4-Methoxytribenzylamine[a]	Benzyl bromide	4-Methoxydibenzylamine	190 (0.4)	65.24	5.97	3.46	65.18	6.20	3.59	84
4-Chlorotribenzylamine[b]	Benzyl bromide	4-Chlorodibenzylamine	160 (0.3)	61.59	5.17	3.42	61.63	5.24	3.59	80
3-Methyltribenzylamine[c]	Benzyl bromide	3-Methyldibenzylamine	171 (0.1)	67.92	6.22	3.60	68.09	6.35	3.60	84
4-Nitrotribenzylamine	4-Nitrobenzyl bromide	Dibenzylamine	55–56	75.97	6.07	8.44	75.82	6.01	8.45	80
N-(Cyclohexylmethyl)-dibenzylamine	Benzyl bromide	N-(Cyclohexylmethyl)benzylamine	47–50	86.08	9.29		86.19	9.25		71
N,N-Dibenzyl-α-phenyl-propylamine	Benzyl chloride	N-Benzyl-α-phenyl-propylamine	144 (0.06)	87.69	8.00	4.45	87.55	8.11	4.48	
α-Carbethoxytribenzylamine	Ethyl α-bromophenyl-acetate	Dibenzylamine	175 (0.075)	80.20	7.02	3.90	79.91	6.88	3.93	66

[a-c] Analyzed as HBF₄ salts; mp *a*, 150–151°; *b*, 173–174°; *c*, 179–181°.

bright yellow crystals from ether, mp 104–105°. N,N-Dibenzyl-*p*-anisidine,[24] mp 81–82°, was prepared similarly.

Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.47; H, 5.79; N. 8.05. Found: C, 72.36; H, 5.98; N, 8.05.

α-Cyanotribenzylamine. A solution of 15.9 g (0.15 mole) of benzaldehyde and 50 ml of glacial acetic acid was stirred while 30 g (0.15 mole) of dibenzylamine was added. After the exothermic reaction had subsided, 10 g (0.15 mole) of potassium cyanide dissolved in 20 ml of water was added dropwise. Upon cooling, an oil separated and solidified; it was recrystallized from ethanol to give 46.5 g (97%) of α-cyanotribenzylamine, mp 103–104°. The infrared spectrum (Nujol mull) showed no absorption in the region of the C≡N stretching frequency, but such behavior is not uncommon for α-amino and α-hydroxy nitriles.

Anal. Calcd. for $C_{22}H_{20}N_2$: C, 84.69; H, 6.46; N, 8.78. Found: C, 84.74; H, 6.54; N, 8.81.

The General Procedure for the Nitrosation of Tertiary Amines in Aqueous Acetic Acid. The amine (0.1 mole) was dissolved in 500 ml of 60% aqueous acetic acid buffered to pH 4–5 with 68 g of sodium acetate. The mixture was stirred and heated on the steam bath to a temperature of approximately 90°. At this point the dropwise addition of 69 g (1 mole) of sodium nitrite dissolved in 100 ml of water was commenced and allowed to continue with heating over a period of about 45 min. After heating for an additional 2 hr, the mixture was cooled, poured into 200 ml of cold water, and extracted with three 200-ml portions of ether. The combined ether extracts were washed with 10% potassium carbonate until the aqueous layer was basic, then with saturated salt solution, and finally were dried over anhydrous sodium sulfate.

Assay of the Nitrosation Mixtures. The ethereal mixture of products from the nitrosation was concentrated, and a small sample was taken from vpc analysis. The remaining solution was completely stripped of ether, and the carbonyl products were fractionally distilled at aspirator pressure through an efficient column. The carbonyl products were weighed and characterized by a comparison of their infrared spectra and the melting points of their 2,4-dinitrophenylhydrazone or semicarbazone derivatives with those of the authentic compounds.

The distillation residue containing the nitrosamine portion of the reaction mixture was refluxed for 60 hr in 300 ml of ethanol containing 12 g of urea and 50 ml of concentrated hydrochloric acid. The alcohol was removed (aspirator), and the residual salts were basified with ammonium hydroxide. The mixture was extracted with three 125-ml portions of ether, and the combined extracts were washed with saturated sodium chloride solution and dried over anhydrous potassium carbonate. The ether was stripped off, and the oily mixture of secondary amines was fractionally distilled under reduced pressure. The amines were weighed and then converted to their hydrochlorides for characterization.

The results of the assay are shown in Table II. The low vapor pressure and high polarity of the nitrosamines did not allow their analysis by vpc. The vpc analysis of the carbonyl compounds

was carried out with a Barber-Coleman Model 8000-2600 J 5 gas chromatograph (Sr⁹⁰ ionization detector) with a 6-ft Ucon oil-on-Firebrick column at a temperature of 145°. Retention times were compared with authentic samples and integration was performed with the aid of a planimeter.

Isolation of the Amines from the Nitrosation of 4-Methoxytribenzylamine. When it was found that the treatment of 4-methoxy-dibenzylnitrosamine with alcoholic hydrochloric acid brought about partial cleavage of the methoxyl group, the following procedure was adopted. The aldehydes were distilled off at reduced pressure and set aside. The nitrosamines were dissolved in 400 ml of 95% ethanol and reduced by the portionwise addition of 600 g of 2.5% sodium amalgam and 40 g of acetic acid over 1 hr. The mercury was removed and the alcohol stripped off at reduced pressure. The resulting residue was taken up in ammonium hydroxide and extracted with several portions of ether. The combined ether extracts were dried over anhydrous potassium carbonate, the ether was evaporated, and the mixture of amines was fractionated.

Treatment of Tribenzylamine with a Limited Amount of Nitrous Acid. Tribenzylamine[25] (28 g, 0.1 mole) was treated with 7 g (0.1 mole) of sodium nitrite by the general procedure. The crude product mixture was distilled to give a small quantity of benzaldehyde and 8.49 g (38%) of dibenzylnitrosamine, bp 140–143° (0.1 mm), mp 59–60° (lit.[26] mp 59°). No other material distilled. The pot residue was taken up in ethanol and recrystallized to give 14.5 g (51.6%) of tribenzylamine, mp 90–91° (reported 90°). The material balance on the amines was 89.6%.

Nitrosation of 4-Nitrotribenzylamine. After the standard nitrosation procedures, the ether was distilled off and the benzaldehyde (3 g, 0.028 mole) distilled at 70–80° (17 mm). The remaining viscous oil was sublimed at 0.5 mm to give *p*-nitrobenzaldehyde (1.62 g, 0.011 mole). The residue was refluxed with 50 ml of concentrated hydrochloric acid and 12 g of urea in 250 ml of ethanol for 36 hr. The alcohol was removed (aspirator), and the residue was treated with excess ammonium hydroxide. The mixture was extracted with ether, and the extracts were dried over anhydrous sodium sulfate. The ether was evaporated, and the resulting oil was distilled at 140° (0.7 mm) to give dibenzylamine (2.96 g, 0.015 mole). The residue was dissolved in anhydrous ether, and hydrogen chloride was bubbled through until precipitation ceased. The precipitate (10.48 g, 0.038 mole) was filtered off, mp 244–248°, undepressed by an authentic sample of 4-nitrodibenzylamine hydrochloride. The filtrate was treated with more hydrogen chloride, and another precipitate formed. This material (6.06 g, 0.0165 mole) melted at 190–194°, undepressed by authentic 4-nitrotribenzylamine hydrochloride.

Nitrosation at Different Temperatures. The amines nitrosated were 3-methyl-, 4-chloro-, and 4-methoxytribenzylamine. A stock solution of the solvent for the experiment was prepared by mixing 68 g of sodium acetate, 300 ml of glacial acetic acid, and 200 ml of water. Each nitrosation was carried out at 62.8, 81.3, and 93.8° in a constant-temperature bath. A mixture of 0.01 mole of the

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amine and 50 ml of solvent was stirred together for 1 hr prior to the addition of 10 ml of 7.7 M sodium nitrite solution, which was withdrawn with a syringe from a flask immersed in the constant temperature bath. After 1 hr the reaction mixture was poured into 150 ml of water and extracted with three 30-ml portions of ether, which were combined and washed with 10% potassium carbonate solution, and then dried over anhydrous sodium sulfate.

The extracts were analyzed for aldehydes by vapor phase chromatography; analysis of known mixtures showed that the method was correct to within 1%. (Known mixtures of benzaldehyde and the substituted aldehyde gave the following analyses: p-chloro, molar ratio (mr) 1.65, area ratio (ar) 1.58; 3-methyl, mr 0.578, ar 0.576; 4-methoxy, mr 0.818, ar 0.830.) The area ratio of the vpc peaks for a given run divided into a statistical factor of two gives k_2/k_1, values for which are given in Table II.

Nitrosation of N-(Cyclohexylmethyl)dibenzylamine. N-(Cyclohexylmethyl)dibenzylamine (21 g, 0.075 mole) was nitrosated by the general procedure. Analysis of the product mixture by vpc showed benzaldehyde to be the only carbonyl-containing product. Distillation of the reaction mixture produced benzaldehyde (4 g, 50%) and 14 g of a yellow oil, bp 125° (0.02 mm), presumed to be N-(cyclohexylmethyl)benzylnitrosamine. The oil was treated with ethanolic hydrochloric acid in the manner described previously. A single substance, distilling at 103° (0.2 mm), was obtained; its infrared spectrum and the melting point of the benzenesulfonamide (75°) were identical with those from N-(cyclohexylmethyl)benzylamine.

Nitrosation of N,N-Dibenzyl-1-phenylpropylamine. N,N-Dibenzyl-1-phenylpropylamine (31 g, 0.1 mole) was nitrosated by the general procedure. Analysis by vpc showed a ratio of 1.7% benzophenone to 98.3% benzaldehyde. Distillation produced only benzaldehyde (8.1 g, 80%); after the denitrosation treatment, N-benzyl-1-phenylpropylamine (19 g, 82%) was the only nitrogen-containing product isolated (identified by infrared comparison).

Nitrosation of N,N-Diethyl-1-phenylethylamine. A mixture of 35 g (0.2 mole) of N,N-diethyl-1-phenylethylamine[27] and 200 ml of buffered 60% acetic acid solution was heated on a steam bath with stirring while 110 ml of 7.7 M sodium nitrate solution was added over a 45-min period; an ice-cooled trap containing ethanol was connected to the top of the reflux condenser on the apparatus. The mixture was stirred for an additional 2 hr, cooled, poured into 300 ml of water, and extracted with three 100-ml portions of chloroform, which were combined and washed with water, dilute sodium hydroxide solution, and saturated salt solution. After drying over anhydrous sodium sulfate, the chloroform was stripped off, and the residue was taken up in dry n-butyl ether. A stream of anhydrous hydrogen chloride was bubbled through while the solution was heated on the steam bath. A precipitate formed gradually and was removed by filtration after 30 min. The filtrate was washed with dilute base, dried over sodium sulfate, and distilled to give in addition to n-butyl ether, 1 g (3%) of acetophenone, which gave a 2,4-dinitrophenylhydrazone, mp 248° (lit.[28] mp 249–250°).

The precipitate was treated with aqueous base and extracted with ether. Distillation of the dried extracts gave a low-boiling fraction, which when treated with picric acid produced a picrate melting at 154°, undepressed by authentic diethylammonium picrate. Further distillation gave 6 g (18.5%) of N-ethyl-1-phenylethylamine, bp 85–90° (15 mm) (hydrochloride mp 201°, undepressed by an authentic sample).

The ethanol in the trap gave a small amount of acetaldehyde 2,4-dinitrophenylhydrazone, mp 145–146° (undepressed by an authentic sample).

In a separate experiment where the work-up was carried out as described in the general procedure, 0.0761 mole (38%) of N-ethyl-1-phenylethylamine was obtained from 0.2 mole of N,N-diethyl-1-phenylethylamine.

Nitrosation of N,N-Dibenzyl-4-chloroaniline. N,N-Dibenzyl-4-chloroaniline (32 g, 0.1 mole) was nitrosated by the general procedure, and the drowned reaction mixture was extracted with three 100-ml portions of chloroform. The combined extracts were washed with water, 10% potassium carbonate solution, and saturated salt solution, dried over anhydrous sodium sulfate, and distilled to give 4.2 g (42%) of benzaldehyde, bp 70–75° (20 mm).

The residue from the distillation was poured while hot into 95% ethanol and cooled to give yellow crystals of N-benzyl-N-nitroso-4-

chloroaniline. This material (15 g, 62%), after drying had mp 57°, alone or mixed with authentic material.[29]

In an experiment carried out at 50–60°, about 60% of the starting amine was isolated unchanged in addition to the nitrosation products.

Nitrosation of N,N-Dibenzyl-p-anisidine. The drowned reaction mixture from the nitrosation of 30 g of N,N-dibenzyl-p-anisidine (0.1 mole) was extracted with three 200-ml portions of chloroform. The combined extracts were washed with water, 10% potassium carbonate solution, and saturated salt solution, dried over sodium sulfate, and evaporated; the oily residue was divided into two equal portions.

The first portion gave only benzaldehyde (2 g), bp 70–75° (20 mm), on distillation, identified by its infrared spectrum and its 2,4-dinitrophenylhydrazone, mp 235–237°, undepressed by an authentic sample.

The second portion was taken up in ethanol and cooled to give yellow crystals (13 g), melting range 70–98°. Chromatography of a 5-g sample on 200 g of alumina gave two fractions: the first eluted with 10% benzene–petroleum ether mixture, consisted of 3.28 g (49.5%) of N,N-dibenzyl-4-methoxy-2-nitroaniline, mp 104–105°, undepressed by an authentic sample; the second, eluted with benzene, consisted of 1.70 g (37%) of N-nitroso-N-benzyl-p-anisidine, mp 77–78°, undepressed by authentic sample.[30]

In an experiment carried out below 70°, N,N-dibenzyl-2-nitro-4-methoxyaniline (21 g, 61%) was the only product obtained from 30 g of the starting amine.

Nitrosation of N,N-Dibenzyl-p-toluidine. N,N-Dibenzyl-p-toluidine[31] (28 g, 0.1 mole) was nitrosated by the general procedure and worked up as described for N,N-dibenzyl-p-anisidine. Benzaldehyde could be isolated from one portion of product mixture; the second portion, which could not be induced to crystallize, was chromatographed on alumina to give three fractions. The first and major fraction, eluted with 20% benzene–petroleum ether, was a dark red oil (3.5 g), which was distilled to give N-benzyl-p-toluidine (1.08 g), bp 116° (0.8 mm), identical with an authentic sample.[21] The distillation residue would not crystallize.

The second fraction (3 g), eluted with 50% benzene–petroleum ether, consisted of N-nitroso-N-benzyl-p-toluidine, mp 50–52°, identical with an authentic sample.[21]

The third fraction, eluted with 10% ether–benzene, gave 220 mg of a crystalline compound, mp 107–108°, whose analysis suggested the empirical formula $C_{14}H_{13}N_3$. Its infrared spectrum showed no N–H absorption, but the ultraviolet spectrum (in methanol) showed two peaks [λ_{max} 2575 (ϵ 7.81 × 10³) and 290 mμ (ϵ 4.56 × 10³)] characteristic of the N-substituted benzotriazole system.[32] This compound is presumed to be 5-methyl-1-benzylbenzotriazole (lit.[33] mp 102–103°).

Anal. Calcd for $C_{14}H_{13}N_3$: C, 75.40; H, 5.88; N, 18.84. Found: 75.27; H, 6.00; N, 18.82.

Nitrosation of N,N-Diethylbenzylamine. N,N-Diethylbenzylamine[34] (24 g, 0.15 mole) was nitrosated by the general procedure. Distillation of the reaction mixture gave 8.34 g of a mixture of benzaldehyde and diethylnitrosamine, bp 80–90° (25 mm), and 13.76 g (57%) of N-nitroso-N-ethylbenzylamine,[7] bp 150–156° (25 mm) with semicarbazide hydrochloride solution and pyridine to give 4 g (16%) of benzaldehyde semicarbazone, mp 222°.

Nitrosation of α-Carboethoxytribenzylamine. α-Carboethoxytribenzylamine (3.49 g, 0.01 mole) was dissolved in 70 ml of glacial acetic acid and stirred at 90° on the steam bath while 20 ml of 7.7 M sodium nitrite solution was added over a 0.5-hr period. After stirring with heating for 1 hr, the reaction mixture was poured into 300 ml of water and extracted with three 50-ml portions of ether. The extracts were combined and washed with dilute sodium carbonate solution, water, and saturated salt solution, and were dried over anhydrous sodium sulfate. Analysis of the reaction mixture by vpc showed only benzaldehyde and no ethyl phenylglyoxalate. Attempts to characterize the nitrogen-containing product from this and repeated reactions were unsuccessful, owing to decomposition.

Nitrosation of α-Methyltribenzylamine. α-Methyltribenzylamine[35] (0.01 mole) was nitrosated in the manner described for

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α-carboethoxytribenzylamine. Analysis by vpc showed that the carbonyl portion of the reaction mixture contained benzaldehyde and acetophenone in the ratio 92:8. The nitrosamines would not come off the column.

Nitrosation of Ethyl α-Diethylaminophenylacetate. Ethyl α-diethylaminophenylacetate[36] (23.5 g, 0.1 mole) was nitrosated by the general procedure. Distillation of the extracts from the drowned reaction mixture gave 1.71 g of diethylnitrosamine (which, being very water-soluble, was not completely extracted from the reaction mixture), 2.91 g (16.4%) of ethyl phenylglyoxalate distilling at 56° (0.2 mm) characterized by comparison of an infrared spectrum with the authentic material), and 13.42 g (57%) of ethyl α-(N-ethylnitrosamino)phenylacetate, bp 100–105° (0.3 mm). The last substance gave a positive Liebermann nitroso test and was further characterized by its infrared and nmr spectra, which were entirely consistent with the assigned structure, in which restricted rotation about the N–N bond is present (e.g., two singlets, approximately 0.5 proton each, at τ 3.5 and 4.0, corresponding to the α hydrogen of the phenylacetate moiety, and doubled signals assignable to the N-ethyl group, partly overlapping the multiplets of the O-ethyl group).

Attempted Nitrosation of N,N-Dibenzyl-4-nitroaniline. N,N-Dibenzyl-4-nitroaniline[37] (16 g, 0.05 mole) was heated in 200 ml of glacial acetic acid with stirring on a steam bath while 35 g of sodium nitrite in 50 ml of water was slowly added. After 4 hr of heating, the cooled mixture deposited yellow crystals (15.8 g, 99%), mp 131–132°, undepressed by starting amine.

Attempted Nitrosation of Quinuclidine. A solution of 50 ml of 60% acetic acid solution, 5.8 g of sodium acetate, and 0.9 g of quinuclidine[38] was stirred and heated on a steam bath while 6 ml of 7.7 M sodium nitrite solution was slowly added. Stirring and heating were continued for 1 hr, and the mixture was then cooled, carefully basified with 10% sodium hydroxide solution, and extracted with three 50-ml portions of ether. The combined extracts were dried over anhydrous sodium sulfate, and hydrogen chloride was bubbled in. The precipitated hydrochloride weighed 1.1 g, (92%), mp >330°; a portion was basified and converted to the picrate, mp 281–282°, undepressed by quinuclidine picrate.

Attempted Nitrosation of Tribenzylmethylammonium Nitrate. A solution of 13.9 g (3.25×10^{-2} mole) of tribenzylmethylammonium iodide[39] in 50% ethanol was added to a water solution of 5.53 g (3.25×10^{-2} mole) of silver nitrate and the silver iodide removed by filtration. Evaporation of the filtrate gave 9.9 g (84%) of tribenzylmethylammonium nitrate (mp 199–200°), which was heated with 100 ml of acetic acid to about 95° on the steam bath, and 35 ml of 7.7 M sodium nitrite solution was then added over a 45-min period. After 2 hr of stirring while heating, the mixture was evaporated to dryness, and the residue was digested with 100 ml of acetone. Evaporation of the acetone solution gave a solid mixture, which was treated with cold water, leaving 7 g of water-insoluble material, mp 195–197° after recrystallization from ethanol, undepressed by a sample of the starting nitrate. No other organic substance (except sodium acetate) could be detected in the other filtrates and solids.

Gases from Nitrosation of Tribenzylamine. The gaseous products from the nitrosation of 1 g (0.64 mmole) of tribenzylamine were swept from the reaction vessel with carbon dioxide into the bottom of a gas-collection tower containing 800 g of potassium permanganate and 200 g of potassium hydroxide in 1200 ml of water. The gas was then transferred to another trap held at liquid nitrogen temperature and distilled from it into the final collection vessel. Mass spectrographic analysis of samples (75 ml, STP) from two identical experiments showed the presence principally of nitrous oxide (mass 44) contaminated with nitric oxide, argon, and nitrogen.

Tribenzylamine Oxide. A solution of tribenzylamine (28 g, 0.1 mole) in 100 ml of glacial acetic acid and 15 ml of 30% hydrogen peroxide was stirred at 60° for 24 hr and then cooled; a pinch of palladium on charcoal was added to decompose the excess peroxide. The solution was filtered and taken to dryness under reduced pressure. Trituration of the resulting white solid with ether gave 27.7 g (94%) of tribenzylamine oxide, mp 138°.

Anal. Calcd for $C_{21}H_{21}NO$: C, 83.15; H, 6.99; N, 4.62. Found: C, 83.04; H, 7.04; N, 4.52.

Nitrosation of Tribenzylamine Oxide. Tribenzylamine oxide (15 g, 0.05 mole) was nitrosated in the same manner described for the tertiary amines in the general procedure. The aqueous reaction mixture was poured into 200 ml of water and extracted with three 75-ml portions of chloroform. The combined extracts were washed with 25% potassium carbonate solution, dried over sodium sulfate, and distilled to give 3 g (48%) of benzaldehyde, bp 65° (13 mm), identified by its infrared spectrum. The residue from the distillation was poured into 50 ml of ethanol and cooled to give 8.23 g (73%) of dibenzylnitrosamine, mp 58°.

In another experiment, tribenzylamine oxide was treated with 1 equiv of nitrous acid, but only starting material and the foregoing nitrosation products could be detected; no tribenzylamine was isolated.

α-d_2-Tribenzylamine. A 1-l. three-necked flask equipped with a pressure-compensated dropping funnel, Trubore stirrer, and a reflux condenser was flamed out twice under evacuation and filled with dry nitrogen. About 300–400 ml of ether was distilled into the flask from lithium aluminum hydride and the flask charged with 2.75 g (0.65 mole) of 98% lithium aluminum deuteride (Metal Hydrides, Inc.). A solution of 30.1 g (0.1 mole) of N,N-dibenzylbenzamide[40] in 80 ml of dry benzene was added dropwise to the stirred mixture over a 75-min period. The stirring mixture was refluxed for 7 hr, cooled, and hydrolyzed with 6 ml of water followed by 4.8 ml of 10% sodium hydroxide. The inorganic salts were removed by filtration and the ether stripped to leave an oil, which solidified upon addition of ethanol. The amine was recrystallized twice from alcohol to give 16.4 g (57%) of α-d_2-tribenzylamine, mp 90–92° (undepressed by an authentic sample).

Nitrosation of α-d_2-Tribenzylamine. α-d_2-Tribenzylamine (14.5 g, 0.05 mole) was nitrosated by the general procedure, except that the temperature was held at 95°. The aqueous mixture from the nitrosation was poured into 300 ml of water and extracted with three 125-ml portions of chloroform. The combined extracts were washed with water, 10% sodium bicarbonate solution, and saturated salt solution. After drying (sodium sulfate), the chloroform was removed under nitrogen, and the residue was distilled to give 3.21 g (58%) of benzaldehyde, bp 64–65° (12 mm). Three samples were prepared for deuterium analysis by diluting 0.2 ml of benzaldehyde to 2 ml, with carbon tetrachloride.

The residue from the distillation was taken up in ethanol and treated with Norit; cooling produced 7.9 g (71%) of dibenzylnitrosamine,[26] mp 58–59°.

Analysis for Deuterium Content. The deuterium content of the starting α-d_2-tribenzylamine was determined by the method of Keston[10a] to be 8.4 mole % (theoretical maximum 9.52%); the percentage of α deuteration is thus $8.4/9.52 \times 100 = 88.5\%$.

The infrared method and data of Wiberg[10b] were used to determine the proportion of deuteriobenzaldehyde in the mixture. The average % transmission at 2070 cm^{-1} for the three samples was 93.93%, which corresponds to 7.8% deuteriobenzaldehyde; at 2100 cm^{-1}, transmission was 92.2%, corresponding to 7.8% deuteriobenzaldehyde.

A confirmation of the percentage of α deuteration in the dibenzylnitrosamine was accomplished by means of nuclear magnetic resonance. Dibenzylnitrosamine shows complex aromatic proton absorption at about τ 2.9, and two singlets at τ 4.88 and 5.45, which arise from the methylene protons. A comparison of the area under the first peak compared with the areas under the peaks for the methylene protons in the deuterated compound gave a rough value of 7% undeuterated dibenzylnitrosamine.

If the starting α-d_2-tribenzylamine were 100% α dideuterated, then the statistical maximum amount of deuteriobenzaldehyde would be 33%. The amine was 88.5% α dideuterated, however, so the statistical maximum deuteriobenzaldehyde content is (33.33) $\times$ (88.5%) = 29.5%, and $k_H/k_D = 29.5/7.8 = 3.78$.

Nitrosation of Tertiary Amines with Nitrosyl Compounds. Apparatus. All the apparatus used in experiments that fall under this general heading was either baked out in an oven at 140° for 24 hr prior to use or flamed out twice while evacuated.

Solvents. Ether and benzene were dried by refluxing over lithium aluminum hydride and were distilled directly into the reaction vessel. Chloroform was purified by extracting with water eight times, drying over calcium chloride, and distilling from calcium chloride. Dimethyl sulfoxide was distilled from and stored over molecular sieves. Petroleum ether (reagent grade) was not purified but used from a freshly opened bottle.

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Reagents. Tribenzylamine[25] was recrystallized from ethanol twice and dried *in vacuo* over phosphorus pentoxide. N-Methylpiperidine was prepared according to Clarke[41] and distilled from sodium prior to use. Nitrosyl chloride was obtained from Olin Matheson Co. and redistilled twice prior to use. Nitrosyl fluoroborate was prepared by the method of Wannagat and Hohlstein[42] and dried over phosphorus pentoxide *in vacuo* prior to use.

Reaction of Tribenzylamine with Nitrosyl Chloride in Benzene. Tribenzylamine (28 g, 0.1 mole) dissolved in 15 ml of benzene was added over a 20-min period to a stirred solution of 25 ml of nitrosyl chloride in 250 ml of benzene held at 6°. After 30 min, the drying tube was removed from the flask, and the mixture was filtered rapidly through a sintered-glass filter. The precipitate was washed with 250 ml of benzene and dried in a vacuum desiccator; weight 20 g. The filtrate was concentrated by distillation of some benzene and nitrosyl chloride. Ether was distilled into the residual solution (50 ml); a second precipitate developed after about 100 ml of ether had distilled. The first precipitate was treated with dilute base and extracted with ether. Concentration of the extracts left an oil which soon solidified; it was recrystallized from alcohol, and then had mp 91°, undepressed by tribenzylamine. The second precipitate was so hygroscopic as to preclude meaningful weighing; when a portion of it was treated with hot water, an oil appeared with dissolution of the solid. The oil was extracted into ether, the ether was removed, and the residue was treated with 2,4-dinitrophenylhydrazine reagent. The resulting orange crystals were recrystallized from xylene, and melted at 233–235°, undepressed by benzaldehyde 2,4-dinitrophenylhydrazone. The water solution from this treatment was concentrated and cooled to yield white crystals, which melted at 255–256°, undepressed by authentic dibenzylamine hydrochloride. No other products could be detected in the other filtrates.

In a second, otherwise similar, experiment, 20 ml of nitrosyl chloride was used in a total volume of 325 ml of benzene, and the benzene filtrate from the original reaction was distilled directly instead of being diluted with ether. There were obtained 2.3 g (22%) of benzaldehyde, bp 70–75° (12 mm), and 4.76 g (21%) of dibenzylnitrosamine, bp 118–122° (0.5 mm), mp 56–57°. The original precipitate was treated with aqueous alkali and then distilled to give 13.7 g (48%) of tribenzylamine, mp 91°, and 3.9 g (20%) of dibenzylamine, bp 88–92° (0.12 mm), hydrochloride mp 255–256°. A third experiment conducted exactly similarly to the second except for the use of only 8 ml of nitrosyl chloride yielded 2.2 g (20%) of benzaldehyde, 1.3 g (5.8%) of dibenzylnitrosamine, 16.55 g (50%)of recovered tribenzylamine, and 2.50 g (13%) of dibenzylamine.

The Reaction of Tribenzylamine with Nitrosyl Fluoroborate in Chloroform. Apparatus. The reaction vessel was an Ace Glass Crystallizer Assembly, consisting of a 100-ml jacketed flask whose bottom contained a fritted glass filter leading to a stopcock. The flask was fitted with a dropping funnel, ground-glass mechanical stirrer, thermometer, and a solvent inlet tube. The reaction flask was cooled by a stream of nitrogen which was passed through a cooling coil immersed in liquid air and into the jacket of the flask.

A. At Room Temperature. A solution of 7.17 g (0.025 mole) of tribenzylamine in 15 ml of chloroform was added dropwise over the course of 45 min to a stirred solution of 2.9 g (0.025 mole) of nitrosyl fluoroborate in 38 ml of chloroform under an atmosphere of nitrogen. Oxides of nitrogen were immediately evolved, and the solution developed a deep red-brown color. After the mixture had stirred for 1.5 hr, 75 ml of petroleum ether was added and a colorless precipitate formed, which was separated by filtration, washed with petroleum ether, and dried *in vacuo* (weight 8.87 g). The filtrates yielded less than 10 mg of material. The precipitate showed infrared bands at 3200 and 1650 cm^{-1} and nmr absorptions (CDCl$_3$) at τ 5.61 (doublet), 4.78, 4.64, 2.62 (two peaks), and 0.57 (singlet). The peak areas of the doublet bore no integral relationship to any of the singlets, but the area under the two singlets at τ 4.64 and 4.78 was four times that under the 0.57 peak.

A 5-g sample of the precipitate was treated with 50 ml of warm water and extracted with ether. Evaporation of the dried ether extract yielded an oil, which was taken up in 30 ml of ethanol and treated with excess 2,4-dinitrophenylhydrazine reagent. A precipitate of 690 mg (18.4%) of benzaldehyde 2,4-dinitrophenylhydrazone, mp 236°, formed (melting point undepressed by an authentic sample). The aqueous extract of the solid was basified and extracted with ether. Evaporation of the dried ether solution left 2.555 g of an oil, which was found by gas–liquid chromatography to be a mixture of 36% of dibenzylamine and 64% of tribenzylamine. Crystallization from ethanol gave 1.307 g (32.3%) of tribenzylamine, mp 90°. The filtrate was evaporated to an oil, taken up in ether, and treated with dry hydrogen chloride to give 0.607 g of crude hydrochloride, which was recrystallized from ethanol to give 268 mg (9.67%) of dibenzylamine hydrochloride, mp 256°.

Another 2-g sample of the reaction product was placed in 20 ml of water with 5 ml of ethanol and 1 g of sodium hydroxide and heated briefly. The mixture was extracted with five 30-ml portions of ether. The dried ether solution was found by glpc to contain a mixture of 22.4% of benzaldehyde, 29.1% of dibenzylamine, and 48.5% of tribenzylamine; evaporation of the ether left 1.43 g of mixture.

B. At −50 to −55°. The reaction flask was charged with 2.9 g (0.025 mole) of nitrosyl fluoroborate and 38 ml of freshly distilled chloroform and flushed with dry nitrogen; a positive pressure of nitrogen was maintained for the duration of the experiment. The stirrer was started and part of the nitrosyl fluoroborate dissolved. The flask was cooled to −50 to −55°, and a solution of 7.17 g (0.025 mole) of tribenzylamine in 15 ml of chloroform was added dropwise over a 0.5-hr period. A reddish precipitate formed and the mixture was stirred at −50° for an additional 1.5 hr. The solid was rapidly filtered from the chloroform solution and quickly washed with 50 ml of chloroform in two portions at −50° (combined filtrates A). The solid was then suspended in 50 ml of cold petroleum ether and allowed to warm to room temperature with stirring. Evolution of oxides of nitrogen was noted during this time. The resulting white solid was filtered, washed with petroleum ether, and dried *in vacuo*; weight 3.98 g (combined filtrates B).

The white solid had nmr and infrared spectra similar to those obtained from the reaction at room temperature. A 2-g sample was hydrolyzed with alcoholic sodium hydroxide as previously described. The oil obtained weighed 805 mg, and was found by glpc to be a mixture of 16.8% of benzaldehyde, 15.4% of dibenzylamine, and 67.8% of tribenzylamine.

Filtrate A was poured into 300 ml of petroleum ether, precipitating 1.175 g of a solid which, upon treatment with base and extraction into ether, yielded 751 mg of an oily mixture of dibenzylamine (3.3%) and tribenzylamine (96.7%) (benzaldehyde, if present, could not be detected in small amounts, owing to the proximity of its glpc peak to the solvent peak). The petroleum ether filtrate gave 4.714 g of unreacted tribenzylamine, mp 89°, upon evaporation.

Filtrate B yielded only a 10-mg residue when evaporated.

Gas–liquid chromatography for the nitrosyl fluoroborate experiments was performed on a MicroTek 2000 R gas chromatograph equipped with a thermal conductivity detector. All analyses were done on a 4-ft column of 10% S.E. 30 on Chromosorb. The column temperature was held at 175° for 4 min after injection of the sample and then raised to 230° at a rate of 50°/min. The flow rate was 100 cc/min, and the inlet and detector temperatures were 300 and 275°, respectively. Under these conditions the retention times of the authentic substances were: benzaldehyde, 0.5 min; dibenzylamine, 4.8 min; and tribenzylamine, 10.0 min.

Formation of α-Cyanotribenzylamine from Tribenzylamine. Tribenzylamine (14.3 g, 0.05 mole) was treated with 5.8 g of nitrosyl fluoroborate at room temperature as described above, except that the solid product was not dried but was dissolved in 150 ml of dimethyl sulfoxide, to which 6 g of potassium cyanide was added, and the mixture was then stirred overnight. The mixture was poured into 600 ml of water and extracted with three 150-ml portions of ether. The combined extracts were washed with water and saturated salt solution. The ether was stripped off after drying over sodium sulfate, and the resulting oil was taken up in ethanol. Upon cooling, a solid, mp 69–81°, separated; it was recrystallized four times from petroleum ether (bp 60–75°) to give 10 g (0.032 mole, 64%) of α-cyanotribenzylamine, mp 102.5–104°, undepressed by an authentic sample.

Hydrolysis of the Red Tribenzylamine–Nitrosyl Fluoroborate Adduct. The red precipitate was prepared as described above, except that it was covered with 150 ml of petroleum ether and the temperature was held at −45° for the remainder of the experiment. A dropping funnel was charged with 25 ml of precooled 16.7% alcoholic potassium hydroxide solution, which was slowly added to the cold, stirred mixture. The mixture was stirred for 30 min after the addition was complete, and was then allowed to come to

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room temperature with stirring. The mixture was then poured into a separatory funnel containing water and ether and extracted with ether. The aqueous portion was diluted up to 1 l. with distilled water and analyzed for nitrite ion using the method of Kolthoff and Belcher.[43] The average molarity of nitrite ion of the solution from a number of determinations was 0.0140. The organic layer from the extraction was stripped of solvent, giving tribenzylamine (5.2 g, 36%, 0.0181 mole), melting at 90°, undepressed by an authentic sample. The tribenzylamine to nitrite ion ratio was thus 1.3/1. In a duplicate experiment, it was determined (more reliably it is believed) that the aqueous solution was 0.013 M in nitrite ion, and the organic layer yielded 3.55 g (0.0128 mole) of tribenzylamine. The tribenzylamine to nitrite ion ratio was 0.985/1.

Lithium Aluminum Hydride Reduction of the Tribenzylamine–Nitrosyl Fluoroborate Adduct. The reddish adduct was prepared at −45° as described, except that it was covered with about 200 ml of dry ether. While the temperature was held at a −45° and the mixture stirred, 60 ml of a saturated solution of lithium aluminum hydride in ether (about 0.75 M) was added slowly. The characteristic red color of the adduct slowly disappeared and a white suspension resulted; it was stirred at −45° for 90 min, then warmed to −20° and hydrolyzed carefully with 6 ml of water followed by 4.8 ml of 10% sodium hydroxide solution. The stirring mixture was allowed to warm to room temperature, and the precipitate was filtered off. The ether was removed from the filtrate by distillation, and the residue was taken up in ethanol and cooled to give some tribenzylamine.

The precipitate from the filtration was digested with five successive 50-ml portions of absolute ethanol, evaporation of which left nothing.

In another experiment, the mixture that resulted from the hydride addition was allowed to warm to room temperature prior to hydrolysis, but the results were the same.

Reaction of N-Methylpiperidine with Nitrosyl Fluoroborate in Ether at −45°; Treatment of the Product with Lithium Aluminum Hydride. A flask fitted with a drying tube, stirrer, and dropping funnel containing 9.9 g (0.1 mole) of N-methylpiperidine was charged with 100 ml of ether and cooled to −45°. Nitrosyl fluoroborate (11.8 g, 0.1 mole) was then added, and the mixture was stirred for 15 min prior to the dropwise addition of the N-methylpiperidine. After 90 min, the addition was complete and a yellow orange solid had formed. Stirring was continued for an additional 45 min before the dropwise addition of 70 ml of a saturated solution of lithium aluminum hydride in ether was commenced. The mixture was stirred at −45° for 1 hr and then the temperature was allowed to rise. When the temperature in the

flask reached about 0°, a rather exothermic reaction ensued. The mixture was stirred at room temperature for 1 hr and hydrolyzed with 8 ml of water followed by 6 ml of 10% sodium hydride solution.

The salts were removed by filtration and digested in absolute alcohol. Concentration of the alcoholic extracts gave a hygroscopic mass, which was treated with dilute base and extracted with ether. The extract was concentrated and treated with picric acid, yielding a picrate of mp 223–225°, undepressed by N-methylpiperidine picrate. The aqueous mixture from the extraction was brought to pH 7 with dilute fluoroboric acid and then taken to dryness by evaporation in a stream of nitrogen while heating on the steam bath. Digestion of the resulting salts with alcohol extracted nothing.

The ethereal solution from the reaction was dried over sodium sulfate and distilled to give a fraction boiling at about 80–90°, identified as N-methylpiperidine by the melting point of its picrate, 223–225°, undepressed by authentic material. Distillation of the residue at 10 mm pressure gave about 1 g of a liquid boiling at 96–100°. It was unreactive toward gaseous hydrogen chloride, but evolved a flammable gas, presumably hydrogen, when treated with aqueous acid. The liquid, which burned with a green flame, and showed strong infrared absorption in the region 2400–2250 cm⁻¹, was concluded to be the BH₃ adduct of N-methylpiperidine.

Anal. Calcd for $C_6H_{16}NB$: C, 63.90; H, 14.33; N, 12.48. Found: C, 64.14; H, 14.64; N, 12.76.

Another experiment was performed in the same manner except that the mixture was not allowed to warm above −15° after the hydride addition was complete. The mixture was hydrolyzed at this temperature, and then was allowed to warm from −15° to room temperature. The formation of an adduct was not observed, and only N-methylpiperidine could be isolated.

N-Methyl-N,N-pentamethylenehydrazinium fluoroborate was prepared by treating the corresponding iodide[44] with silver fluoroborate in water. The fluoroborate could be reconverted to the iodide by treatment with sodium iodide in alcohol, and melted at 146–150°.

Treatment of N-Methyl-N,N-pentamethylenehydrazinium Fluoroborate with Lithium Aluminum Hydride in Ether. The hydrazinium salt (6.5 g) suspended in 50 ml of ether was cooled to −10°, and the 70 ml of saturated ethereal lithium aluminum hydride solution was slowly added with stirring. After 1 hr the temperature was allowed to rise to 20° and stirring was continued for 1 hr more. The mixture was then refluxed for 30 min, cooled, and hydrolyzed with 6 ml of water followed by 4.8 ml of sodium hydroxide solution. The salts were filtered off and digested in absolute ethanol to give a small amount of the unchanged quaternary hydrazine. The ether solution was distilled to give a small amount of N-methylpiperidine (identified by its picrate) followed by N-methylpiperidine–borane, bp 100° (11 mm), identified by its infrared spectrum.

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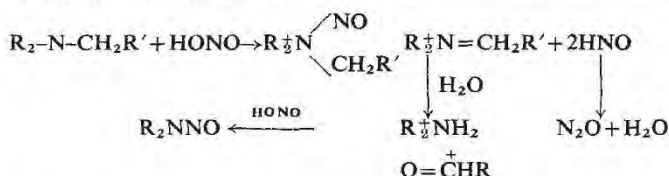(44) L. Knorr, *Ann.*, **221**, 297 (1883).

Exhibit 39

BIOLOGICAL SCIENCES

Formation of N-Nitrosodimethylamine from Naturally Occurring Quaternary Ammonium Compounds and Tertiary Amines

THE potential for the formation of N-nitrosamines in the human environment, either during the processing of foods or *in vivo* from nitrate and/or nitrite, and precursor amines has received considerable attention in recent years. Traditionally only secondary amines were thought to undergo N-nitrosation reactions. While secondary amines *per se* are not common in biological systems, tertiary amines and quaternary ammonium compounds do occur in plant and animal tissue. The possible formation of N-nitrosamines from these compounds must be considered because recent reports have appeared on the nitrosation of trimethylamine[1,2] and trimethylamine oxide[1] in connexion with the possible formation of N-nitrosamines in fish products cured with nitrate. The nitrosative cleavage of tertiary amines is not new and has been described before[3,4].

We are reporting the formation of dimethylnitrosamine (DMNA) from several quaternary ammonium compounds and some of their related tertiary amines in conditions simulating those found in comminuted meat products. The N-containing compounds (4.56 mmol) were reacted with $NaNO_2$ (22.8 mmol) in pH 5.6 buffer for 4 h at 78° C, then extracted with CH_2Cl_2; the extracts were dried with anhydrous Na_2SO_4 and concentrated in a Kuderna-Danish concentrator. DMNA was determined by a Varian aerograph model 1520 gas-liquid chromatograph using FID. The GLC conditions are as follows: column—2 feet × 1/8 inch absorbance 100–120 mesh 'Chromosorb 101'; flow rates (cm^3 min^{-1})—He 102, air 429, H_2 57; temperatures (°C)—detector 218; injector port 185°, column isothermal 150°. A gas-liquid chromatography-mass spectroscopy (GLC-MS) system with a DuPont model 492 mass spectrometer was used for all confirmation of identification. The results are shown in Table 1. DMNA is produced from tetramethylammonium chloride at almost the same level as from trimethylamine. The quaternary ammonium compound is demethylated, as shown by the accumulation of trimethylamine in the absence of nitrite. Smith[4] has proposed a pathway for the N-nitrosation of tertiary amines that appears to be applicable in this case:

$$R_2-N-CH_2R' + HONO \rightarrow R\overset{+}{_2}N\begin{subarray}{l}\nearrow NO \\ \searrow CH_2R'\end{subarray} \quad R\overset{+}{_2}N=CH_2R' + 2HNO$$

$$R_2NNO \xleftarrow{\text{HONO}} R\overset{+}{_2}NH_2 \quad N_2O + H_2O$$

$$O=\overset{+}{C}HR$$

The naturally occurring quaternary ammonium compounds, neurine, carnitine, betaine, choline and acetylcholine, also yield DMNA when reacted with $NaNO_2$ although at a considerably lower level than the tetramethylammonium compound. The results reflect the ability of the quaternary compound to dealkylate to trimethylamine or a mixed amine containing the Me_2N- group and another substituent. N,N-Dimethylglycine and its methyl ester are tertiary amines derived from the rearrangement of betaine in acidic conditions; 2-dimethylaminoethanol and its acetate derivative are similarly related to choline and acetylcholine respectively. The DMNA yield from the mixed alkyl tertiary amines is two to five times that obtained from trimethylamine and 4×10^3 to 2×10^4 times the DMNA formed from the parent quaternary ammonium compounds. In fact, 2-dimethylaminoethyl acetate forms DMNA almost one-half that formed by dimethylamine. It is interesting to note that larger quantities of DMNA are produced from the esters of the quaternary and tertiary compounds than from the parent compounds.

It is significant that DMNA can be formed from quaternary ammonium compounds, albeit at low levels, in the conditions described. We can therefore conclude that although quaternary ammonium compounds may represent a potential source of nitrosamine, the tertiary amines and free secondary amines present in food products are of greater concern.

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Table 1 Formation of N-Nitrosodimethylamine from Naturally Occurring Quaternary Ammonium Compounds and Tertiary Amines

Structure	Name	mg DMNA mol amine*
$(CH_3)_2NH.HCl$	Dimethylamine . HCl	7,108
$(CH_3)_3N.HCl$	Trimethylamine . HCl	643
$(CH_3)_4\overset{+}{N}Cl^-$	Tetramethylammonium chloride	433
$(CH_3)_3\overset{+}{N}-CH=CH_2Cl$	Neurine chloride	133
$(CH_3)_3\overset{+}{N}-CH_2CH_2OAcCl^-$	Acetylcholine chloride	0.897
$(CH_3)_3\overset{+}{N}-CH_2CH_2OHCl^-$	Choline chloride	0.160
$(CH_3)_3\overset{+}{N}-CH_2CO_2^-$	Betaine	0.159
$(CH_3)_3\overset{+}{N}-CH_2CH CH_2CO_2HCl^-$ OH	Carnitine chloride	0.044
$(CH_3)_2N-CH_2CH_2OAc$	2-Dimethylaminoethyl acetate	3,524
$(CH_3)_2N-CH_2CH_2OH$	2-Dimethylamino-ethanol	1,179
$(CH_3)_2N-CH_2CO_2Me$	Methyl ester of N,N-dimethylglycine	3,004
$(CH_3)_2N-CH_2CO_2H$	N,N-Dimethylglycine	1,405

* Quantification based on the use of cyclohexanone as an internal standard.

N-Dimethylnitrosamine in Tobacco Smoke Condensate

THE possible presence of N-nitrosamines in cigarette smoke and/or condensate has been of interest for some years. Druckrey and Preussmann[1] postulated that N-nitrosamines could be produced in burning tobacco, but the difficulty of measuring these compounds prevented the testing of this postulate. We have developed[2] a gas chromatographic method for the selective detection of N-nitrosamines, and now report its application to the analysis of cigarette smoke condensate. We have identified N-dimethylnitrosamine (DMN) in cigarette smoke condensate, and shown that certain tobaccos grown in different conditions vary widely in their content of DMN.

Exhibit 40

Theoretical Investigation of *N*-Nitrosodimethylamine Formation from Nitrosation of Trimethylamine

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Tertiary amines have been demonstrated to be capable of undergoing nitrosative cleavage to produce carcinogenic *N*-nitrosamines. The reaction mechanism of the nitrosation of trimethylamine (TMA) to produce *N*-nitrosodimethylamine (NDMA) was investigated at the CBS-QB3 level of theory. The formation of NDMA from TMA was proposed to be initiated by the formation of an iminium ion, $Me_2N^+{=}CH_2$. Two different mechanisms (NOH elimination mechanism and oxidation abstraction mechanism) leading to $Me_2N^+{=}CH_2$ were investigated, and the oxidation abstraction mechanism was found to be mainly operative. This result indicates that the oxidation abstraction mechanism plays an important role in the nitrosation of both *N,N*-dialkyl aromatic and tertiary aliphatic amines. Starting from the iminium ion, two experimentally proposed mechanisms (pathways 1 and 2) and one new mechanism (pathway 3) were examined. Pathway 1 proposes that the iminium ion undergoes hydrolysis to give dimethylamine (DMA), which then can be further nitrosated to NDMA; pathway 2 proposes that the iminium ion reacts with NO_2^- and forms a neutral intermediate, which then collapses to NDMA. In pathway 3, the iminium ion reacts with N_2O_3 to give NDMA. Calculation results indicate that in aqueous solution pathway 1 is more feasible than pathways 2 and 3; moreover, the transformation from the iminium ion to NDMA is the rate-determining step. This work will be helpful to elucidate the formation mechanisms of the carcinogenic *N*-nitrosamines from the nitrosation of tertiary amines.
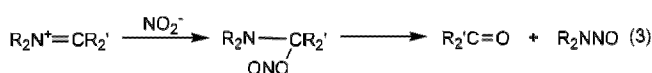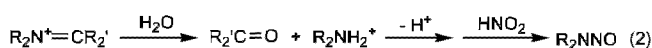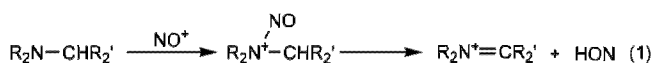
1. Introduction

It is well known that *N*-nitrosamines are a class of undesired industrial and environmental pollutants, many of which are carcinogenic, mutagenic, and teratogenic.[1–7] In particular, *N*-nitrosodimethylamine (NDMA), which is the simplest di-alkylnitrosamine, has been demonstrated to be a potent carcinogen to various organs in animals, including liver, lung, and kidney.[8–12] As reported, NDMA has been found in air, soil, water, food, cosmetics, rubber products, and many other materials.[13–25] Therefore, the U.S. Environmental Protection Agency (U.S. EPA) defined NDMA as a probable human carcinogen.[26]

Because dialkylnitrosamines are of great interest in carcinogenesis, much attention has been focused on their formation mechanism, especially from secondary amines. Consequently, NDMA is generally believed to be formed from the reactions of dimethylamine (DMA) and nitrosating agents, such as N_2O_3, N_2O_4, and ONCl.[27–31] In addition to secondary amines, however, a wide variety of tertiary amines have also been demonstrated to react with nitrous acid to produce *N*-nitrosamines in aqueous solution.[32–44] In view of the ubiquity of tertiary amines, it is significant to understand the formation mechanism of *N*-nitrosamines from them, especially the formation of NDMA. Previous experimental studies revealed that the nitrosation of tertiary amines proceeds via complex mechanisms depending on the structure of the amine, molar ratio of amine to nitrite, temperature, and pH.[34,35,38,42] Different from the nitrosation of secondary amines, it has been found that a relatively weak acid solution and mild warming are generally required for the *N*-nitrosamines formation from tertiary amines.[33,34,45] In addition, an α hydrogen atom is also required to exist in tertiary amine

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for the reaction to proceed.[33] On the basis of experimental results, the reactions were presumed to take place through the elimination of NOH from a nitrosammonium ion to produce an iminium ion $R_2N^+{=}CR_2'$, which then undergoes hydrolysis and further nitrosation (eqs 1 and 2). On the basis of this hypothesis, a viable alternative was then advanced as eq 3, implying the possibility of nucleophilic attack of NO_2^- toward the iminium ion to give the *N*-nitrosamine. Noticeably, the mechanisms described in eqs 1–3 became predominant in subsequent studies on the *N*-nitrosamines formation from normal aliphatic tertiary amines.

Several experimental studies of the reaction of simple aliphatic tertiary amines and nitrite obtained the similar result that the optimum pH for the production of the corresponding *N*-nitrosamines at elevated temperature (100 °C) is about 3.0 to 3.3.[37,38,41,46] It is noteworthy that the formation of nitrosating agent N_2O_3 is facilitated at this acidity. This suggests that a new mechanism rather than the mechanisms proposed as eqs 1–3 might become operative. A kinetic study performed by Ohshima and Kawabata[46] found that the rate of NDMA formation from TMA at pH 3.0 and 100 °C is proportional to the square of nitrite concentration. Therefore, a mechanism similar to eqs 1 and 2 was proposed, where the nitrosating agent

SCHEME 1: Mechanistic Pathway for the Formation of Iminium Ion with the NOH Elimination Mechanism
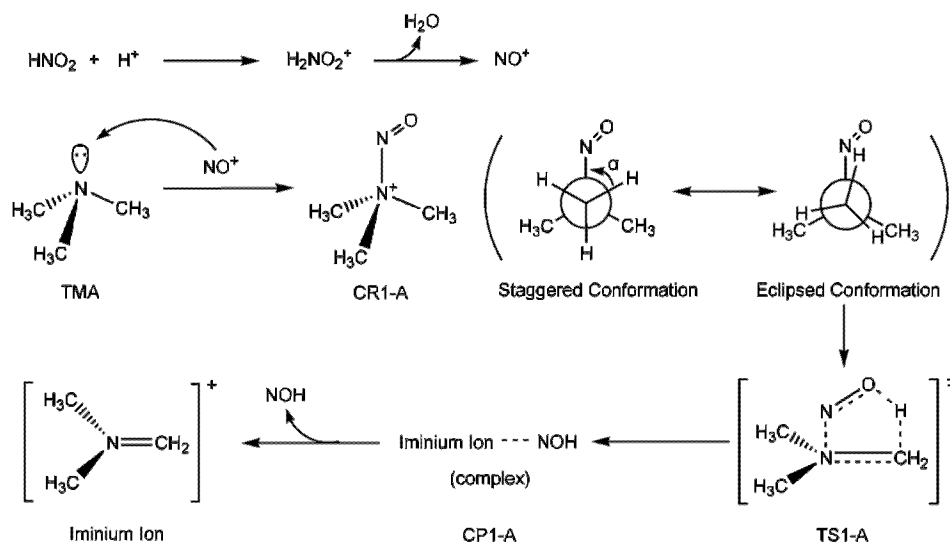
was suggested to be N_2O_3 rather than HNO_2. This hypothesis is reasonable because N_2O_3 has been demonstrated to be a more potent nitrosating agent than HNO_2 in the nitrosation of secondary amines.[30] However, the possibility for the direct nitrosation of the iminium ion by N_2O_3 has not yet been taken into consideration.

In addition, two recent studies[47,48] suggested that the tertiary amine may be an important precursor of carcinogenic NDMA during water disinfection process, and the proposed mechanism also involves the dealkylation of tertiary amine, which is similar to the proposed nitrosation mechanism of tertiary amines. Therefore, the investigation of the nitrosation mechanism of tertiary amines may also provide a useful model to investigate the newly found reaction, which may take place during water treatment.

Few theoretical investigations were found to make contributions to elucidate the reaction mechanism of nitrosation of tertiary amines. To better understand this kind of reaction, the present work is a theoretical research on the detailed mechanistic pathways for the formation of *N*-nitrosamine from the reaction of tertiary amine with nitrite. Trimethylamine (TMA), the simplest trialkylamine and one that can be derived from the ordinary diet,[49-51] has been suggested to be a possible precursor of NDMA,[38,46,52] and so it was selected as the model compound.

2. Theoretical Methods

All structures of reactants, transition states, intermediates, and products were fully optimized by using the B3LYP method (Becke's three-parameter nonlocal exchange functional[53] with the correlation functional of Lee, Yang, and Parr[54]) in conjunction with 6-311+G(d,p) basis set. Vibrational frequencies were also calculated at the same level to characterize the nature of each stationary point. The intrinsic reaction coordinate (IRC)[55] calculations were performed to confirm that every transition state connects the corresponding reactant and product through the minimized-energy pathway. On the basis of the optimized geometries at the B3LYP/6-311+G(d,p) level, reoptimizations of these stationary points were performed with the complete basis set (CBS-QB3) methodology, in which B3LYP density functional theory is combined with the CBSB7 basis set.[56]

Because the proposed mechanisms are expected to take place in aqueous solution, the solvent effect of water on the reactions of NDMA formation from TMA was also taken into account

in this study. On the basis of the optimized geometries obtained at the CBS-QB3 level, the single-point energy calculation was carried out by using the conductorlike polarizable continuum model (CPCM)[57] at the CCSD(T)/6-311+G(d,p) level,[58] denoted as CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7. According to the calculation results from Takano and Houk,[59] the UAKS cavity was used in this study to evaluate the aqueous solvation effects, and the rest of the parameters of the models have been kept as default values from the Gaussian 03 program package.[60] Because the improved density functional PBE1W has been proven to be better than B3LYP method for calculating the energies of water clusters,[61,62] we also investigated the hydrolysis mechanism by using the PBE1W method, and the result will be used to compare with that at the CBS-QB3 level. The absolute energy data are provided in the Supporting Information. All calculations presented in this work were carried out with the Gaussian 03 program package.

3. Results and Discussion

3.1. Formation of the Iminium Ion. Two different pathways were investigated to elucidate the formation of the iminium ion. The first pathway is the mechanism described in eq 1, which involves the formation of a nitrosammonium ion Me_3NNO^+, followed by elimination of NOH to give the iminium ion (NOH elimination mechanism). The second pathway is a new mechanism recently proposed by Loeppky et al.[63] This mechanism involves the oxidation of the tertiary amines to a radical cation by NO^+, followed by the H-abstraction to produce the iminium ion by nitrogen dioxide, NO_2 (oxidation abstraction mechanism). The two mechanisms will be discussed in turn.

3.1.1. NOH Elimination Mechanism. The NOH elimination mechanism involves the formation of a nitrosammonium ion (Me_3NNO^+) and followed by elimination of NOH to give the iminium ion. The detailed reaction pathway is illustrated in Scheme 1, and the fully optimized structures of all stationary points involved in this process are shown in Figure 1. The relative energies are listed in Table 1.

As shown in Scheme 1, TMA first undergoes electrophilic attack of NO^+ to form a nitrosammonium ion, which is a positively charged reactant complex (CR1-A). Figure 1 shows that the N—N bond length and the angle $\angle O-N-N$ in CR1-A were predicted to be 1.884 Å and 111.9°, respectively. As listed in Table 1, the enthalpy change (ΔH) was calculated to be
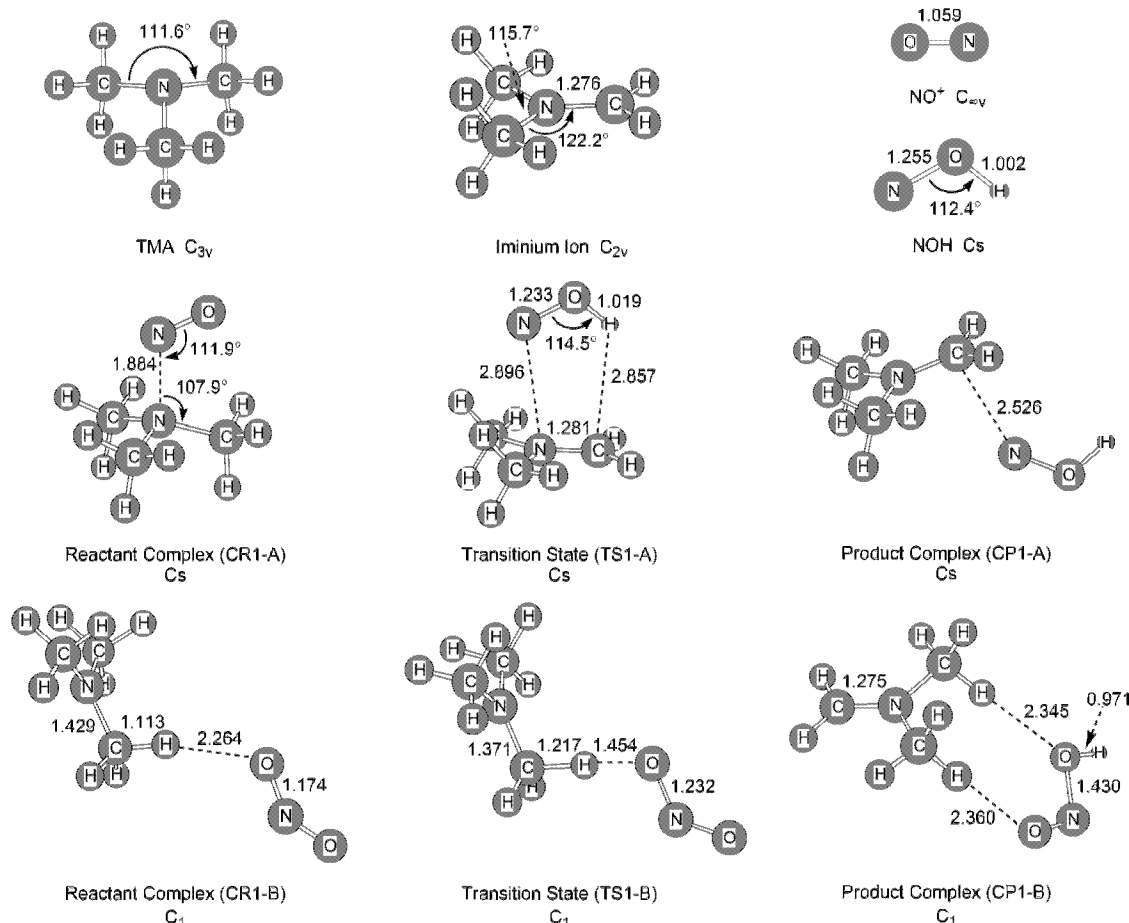
Figure 1. Optimized geometries and main parameters of all stationary points involved in the process of the formation of the iminium ion (distances in angstroms).

TABLE 1: Relative Energies (RE), Enthalpies (RH), and Free Energies (RG) of each Stationary Point Involved in the Formation of the Iminium Ion at the CBS-QB3 Level in the Gas Phase and Aqueous Solution[a,b]

species	RE	RH	RG	RE_w[b]
NOH elimination mechanism				
TMA + NO$^+$	0.00	0.00	0.00	0.00
CR1-A	−58.64	−58.97	−50.33	−20.65
TS1-A	−3.80	−3.96	3.94	32.97
CP1-A	−10.46	−10.14	−3.85	27.66
iminium ion + NOH	−2.20	−1.84	−3.64	29.88
oxidation abstraction mechanism				
TMA$^+$ + NO$_2$	0.00	0.00	0.00	0.00
CR1-B	−27.71	−27.19	−20.05	48.53
TS1-B	17.26	16.78	27.32	39.02
CP1-B	−47.96	−47.98	−39.08	−46.12
iminium ion + HNO$_2$	−40.83	−41.38	−39.30	−45.24

[a] Gas phase: relative energies, enthalpies, and free energies in kilocalories per mole. [b] Aqueous solution: relative energies (RE$_w$) in water at the CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7 level for comparison.

−58.97 kcal/mol, which indicates that the formation of CR1-A is an exothermic process in the gas phase. The change of Gibbs free energies (ΔG) was calculated to be −50.33 kcal/mol, indicating that the formation of CR1-A from TMA and NO$^+$ is thermodynamically favored and can take place spontaneously at 298 K and 1 atm. Then, a cis elimination of NO moiety with a hydrogen atom from an α-carbon in CR1-A occurs to produce

the NOH and iminium ion. Reaction barrier for this elimination was predicted to be 54.84 kcal/mol in the gas phase.

Moreover, Scheme 1 shows that two steps are required for the formation of iminium ion. The first step is the transformation of CR1-A from a staggered conformation to an eclipsed one, which was caused by the rotation of N−C single bond. To characterize the nature of this transformation, an additional calculation of potential energy surface (PES) scan was performed at the B3LYP/CBSB7 level, and the result was illustrated in Figure 2. As shown in the PES, the energy continually increases when changing from the staggered conformation to the eclipsed one, and the energy gap between the two conformations is only 1.51 kcal/mol at the CBS-QB3 level, which indicates that the transformation occurs easily. It is interesting to note that the eclipsed conformation of CR1-A per se is not a stationary point but a transition state on the PES because the imaginary frequency is 155.2i cm^{-1}. The vibrational mode of this imaginary frequency corresponds to a rocking vibration of the methyl group through the rotation of the N−C single bond and connects two identical staggered conformations. In the second step, a five-membered cyclic transition state (TS1-A, 137.7i cm^{-1}) is formed from CR1-A in the eclipsed conformation. As illustrated in Figure 1, the N−N and H−C distances in TS1-A were calculated to be 2.896 and 2.857 Å, respectively. These unexpectedly long distances were possibly caused by the fact that the positively charged TS1-A is an electron deficient species. In addition, only slight differences were found between the geometries of NOH moiety in TS1-A and the fully optimized NOH molecule with C_s point group, as shown in Figure 1. A
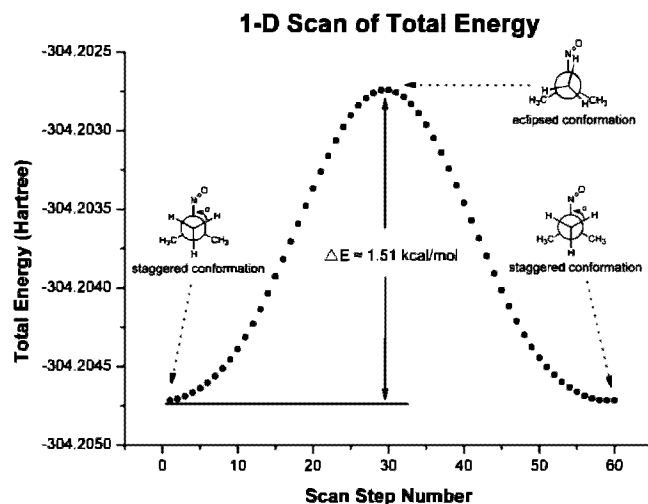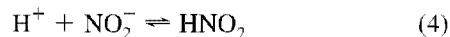
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1-D Scan of Total Energy

Figure 2. Scan plot of potential energy surface (PES) for the transformation of CR1-A from the staggered conformation to the eclipsed conformation at the B3LYP/CBSB7 level.

similar situation can also be observed between the other moiety of TS1-A and the fully optimized iminium ion. Both of the vibrational mode of imaginary frequency and IRC calculations at the B3LYP/6-311+G(d,p) level confirm that this transition state does connect the corresponding reactant complex (CR1-A) and product complex (CP1-A). The energy required to separate the NOH and iminium ion from CP1-A was calculated to be 8.26 kcal/mol, as shown in Table 1.

3.1.2. Oxidation Abstraction Mechanism. The oxidation abstraction mechanism involves the oxidation of the TMA to a radical cation by NO^+ and then followed by the H-abstraction to produce the iminium ion by NO_2. The detailed reaction pathway of this mechanism is illustrated in Scheme 2, and the fully optimized structures of all stationary points involved in this mechanism are shown in Figure 1. The relative energies are listed in Table 1.

As shown in Scheme 2, similar as the case of Scheme 1, TMA first undergoes electrophilic attack of NO^+ to form the nitrosammonium ion (CR1-A). This is followed by the elimination of nitric oxide NO to produce a radical cation, TMA^+. The TMA^+ further undergoes the H-abstraction by the attack of NO_2 to form the iminium ion and HNO_2. The origin of the radical NO_2 is described as eqs 4−6. Two HNO_2 molecules first react to yield N_2O_3, followed by the homolytic dissociation of the N−N bond in N_2O_3 to give NO_2.

$$H^+ + NO_2^- \rightleftharpoons HNO_2 \qquad (4)$$

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O \qquad (5)$$

$$N_2O_3 \rightleftharpoons {}^\cdot NO + {}^\cdot NO_2 \qquad (6)$$

The total energy of the TMA^+ and NO was calculated to be remarkably lower than that of the TMA and NO^+ by 33.51 kcal/mol. This result implies that the oxidation of TMA to TMA^+ by NO^+ is exothermic. It is illustrated in Figure 1 that the main geometrical change for the reaction of TMA^+ and NO_2 is the transfer of a hydrogen atom from the TMA^+ to NO_2. It is noteworthy that the energy barrier of the oxidation abstraction mechanism in the gas phase was calculated to be 44.97 kcal/mol, which is lower than the barrier of the NOH elimination mechanism (54.84 kcal/mol), as shown in Table 1.

It should be noted that both the NOH elimination and oxidation abstraction mechanisms proposed the positively

charged NO^+ as the oxidative species in this article. However, there is still the possibility of the other species such as NO_2 or N_2O_3 as the oxidant. Therefore, what is the oxidative species to amines may be still an open question.

3.2. Formation of NDMA from Iminium Ion. Generally, there are two main reaction mechanisms for the formation of NDMA from the iminium ion. In the first mechanism, the iminium ion undergoes hydrolysis to be degraded to a secondary amine DMA, which can then be further nitrosated to NDMA. In the second mechanism, the iminium ion directly reacts with nitrosating species (NO_2^- or N_2O_3) to produce NDMA. In this study, three pathways were considered. Pathway 1 belongs to the first mechanism, in which the iminium ion undergoes hydrolysis to form DMA. Pathways 2 and 3 belong to the second mechanism, in which the iminium ion undergoes a nucleophilic attack from NO_2^- and N_2O_3, respectively, and then collapses to NDMA. The former two pathways were proposed on the basis of previous experimental results, and the third is a pathway that was put forward on the basis of the fact that N_2O_3 can be formed under the mild acid condition. Detailed discussions for pathways 1, 2, and 3 will be given in turn.

3.2.1. Reaction Pathway 1: Hydrolysis Mechanism. Pathway 1 proposes that the nascent iminium ion undergoes hydrolysis to give a protonated adduct, which then loses a H^+ to give a formaldehyde molecule and DMA, which will be nitrosated finally to NDMA. Two different hydrolysis mechanisms were examined: (A) non-assisted hydrolysis mechanism (only one H_2O molecule involved) and (B) water-assisted hydrolysis mechanism (two H_2O molecules involved). Because the improved density functional PBE1W has been proven to be better than the B3LYP method for calculating the energies of water clusters,[60,61] the hydrolysis mechanism was also investigated by using the PBE1W method, and the result will be used to compare with that at the CBS-QB3 level (Tables 2 and 3). The detailed reaction pathways are illustrated in Schemes 3 and 4. The hybridization change of N and C atoms involved in the mechanism is described in Figure 3, and the fully optimized structures of all stationary points involved in this mechanism are shown in Figures 4 and 5. The relative energies are listed in Tables 2 and 3.

A. Non-Assisted Hydrolysis Mechanism. As illustrated in Scheme 3, a reactant complex (CR2-A) composed of the nascent iminium ion and one H_2O molecule is first formed. Table 2 shows that the enthalpy change ΔH for the formation of CR2-A was calculated as −9.21 kcal/mol, which predicts an exothermic process. As illustrated in Figure 3, in CR2-A, the N and C atoms are sp^2 hybridized, and the p orbitals of the N and C atoms that are perpendicular to the planar iminium ion overlap each other and form a stable conjugated system. The iminium ion moiety shows a planar structure in which the dihedral angles $D(C-N-C-C)$ and $D(H-C-H-N)$ are 178.5 and 178.7° (Figure 4), respectively.

Further approach of the iminium ion and H_2O leads to the formation of a four-membered cyclic transition state (TS2-A, 1374.1i cm^{-1}). In this process, as shown in Figure 4, the O−C bond length decreases from 2.549 to 1.513 Å, whereas the distance between H and N atoms reduces from 3.786 to 1.478 Å, which indicates the formation of C−O and N−H bonds. H−O and N−C bond lengths are increased by 0.182 and 0.177 Å, respectively, which indicates the cleavage of these two bonds. The dihedral angles of $D(C-N-C-C)$ and $D(H-C-H-N)$ reduce to 134.8 and 135.2° when changing from CR2-A to TS2-A, respectively, which implies that the original sp^2 hybridized N and C atoms in CR2-A are going to convert to sp^3

NDMA Formation from Nitrosation of TMA

SCHEME 2: Mechanistic Pathway for the Formation of Iminium Ion with the Oxidation Abstraction Mechanism
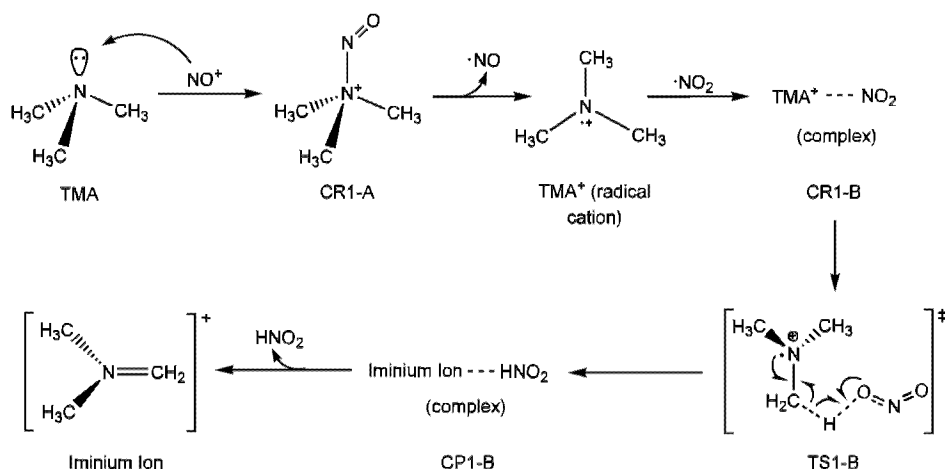
TABLE 2: Relative Energies (RE), Enthalpies (RH), and Free Energies (RG) of Each Stationary Point Involved in the Non-Assisted Hydrolysis Mechanism at the CBS-QB3 Level in the Gas Phase and Aqueous Solution[a,b,c]

species	RE	RH	RG	RE_w[b]	RE_w[c]
iminium ion + H_2O	0.00	0.00	0.00	0.00	0.00
CR2-A	−9.33	−9.21	−3.01	−3.21	−3.24
TS2-A	30.88	29.20	40.34	29.63	29.39
CP2-A	−6.99	−8.24	1.68	−14.42	−16.28

[a] Gas phase: relative energies, enthalpies, and free energies in kilocalories per mole. [b] Aqueous solution: relative energies (RE_w) in water at the CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7 level for comparison. [c] Aqueous solution: relative energies (RE_w) in water at the CPCM-CCSD(T)/6-311+G(d,p)//PBE1W/CBSB7 level for comparison.

TABLE 3: Relative Energies (RE), Enthalpies (RH), and Free Energies (RG) of Each Stationary Point Involved in the Water-Assisted Hydrolysis Mechanism at the CBS-QB3 Level in the Gas Phase and Aqueous Solution[a,b,c]

species	RE	RH	RG	RE_w[b]	RE_w[c]
iminium ion + $2H_2O$	0.00	0.00	0.00	0.00	0.00
CR2-B	−17.44	−18.22	−1.87	−6.83	−5.16
TS2-B	5.97	3.31	24.64	8.47	8.24
CP2-B	−21.90	−23.52	−4.86	−19.36	−19.18

[a] Gas phase: relative energies, enthalpies, and free energies in kilocalories per mole. [b] Aqueous solution: relative energies (RE_w) in water at the CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7 level for comparison. [c] Aqueous solution: relative energies (RE_w) in water at the CPCM-CCSD(T)/6-311+G(d,p)//PBE1W/CBSB7 level for comparison.

hybridization in the process, as illustrated in Figure 3. The conversion of hybridization makes the N and C atoms more capable of accepting the H and O atoms of the H_2O molecule, respectively. In fact, the hybridization of N and C atoms in TS2-A is more like a middle state between sp^2 and sp^3, and further decreased $D(C-N-C-C)$ and $D(H-C-H-N)$ can be observed in the product complex (CP2-A) when compared with that of TS2-A (Figure 4).

As shown in Table 2, the energy barrier for this reaction was calculated to be 40.21 kcal/mol in the gas phase. This relatively high barrier can be rationalized by the stable CR2-A and the high-lying four-membered cyclic transition state TS2-A with strong ring strain.

Natural bond orbital (NBO) analysis[64] for CP2-A showed that the highest positively charged position locates at the hydrogen atom H (+0.500 e) adjacent to oxygen atom. Therefore, this hydrogen atom might be expected to easily undergo the attack of nucleophiles in solution. Consequently, the loss of a proton H^+ will give rise to the formaldehyde and DMA, which can be further nitrosated to NDMA by a nitrosating agent, as shown in Scheme 3.

B. Water-Assisted Hydrolysis Mechanism. Two H_2O molecules are required for the water-assisted hydrolysis mechanism in Scheme 4, which is different from the non-assisted hydrolysis mechanism. It is shown that a reactant complex (CR2-B) between the nascent iminium ion and two H_2O molecules is first formed. As shown in Table 3, enthalpy change ΔH for the formation of CR2-B was calculated to be −18.22 kcal/mol, which predicts that this formation is an exothermic process in the gas phase. Similar to the case of CR2-A, in Figure 5, the N and C atoms in CR2-B are sp^2 hybridized because the iminium ion moiety shows a planar structure in which the dihedral angles $D(C-N-C-C)$ and $D(H-C-H-N)$ are 173.7 and 174.1°, respectively. As discussed in the non-assisted hydrolysis mechanism, the planar structure indicates the formation of an intramolecular stable conjugated system.

As shown in Scheme 4, further approach of the iminium ion and H_2O molecules in CR2-B leads to the formation of a six-membered cyclic transition state (TS2-B, 276.5i cm^{-1}). In this process, the O−C bond length decreases from 2.372 to 1.503 Å, whereas the N−C bond length increases from 1.281 to 1.427 Å. The dihedral angles of $D(C-N-C-C)$ and $D(H-C-H-N)$ reduce to 128.5 and 129.8° when changing from CR2-B to TS2-B, respectively, which implies that the original sp^2 hybridized N and C atoms in CR2-B are going to convert to sp^3 hybridization in the process, as illustrated in Figure 5. Further changes following these tendencies give rise to the product complex (CP2-B), in which the O−C bond length decreases to 1.384 Å, whereas the N−C bond length increases to 1.525 Å, and the dihedral angles of $D(C-N-C-C)$ and $D(H-C-H-N)$ reduce to 127.4 and 115.8°, respectively. Similar to the case of Scheme 3, Scheme 4 shows that the loss of a water molecule and a proton H^+ from the nascent CP2-B gives the formaldehyde and DMA, which can be further nitrosated to NDMA by a nitrosating agent. The nitrosation of secondary amines has already been extensively studied,[27−31] and the DMA has been confirmed to be easily nitrosated into NDMA in an acidic nitrite solution.

As shown in Table 3, the energy barrier for water-assisted hydrolysis mechanism was calculated to be 23.41 kcal/mol in the gas phase. It is notable that this barrier is almost half the magnitude of the barrier for the non-assisted hydrolysis mech-

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SCHEME 3: Mechanistic Pathway for the Formation of NDMA with the Non-Assisted Hydrolysis Mechanism of Pathway 1
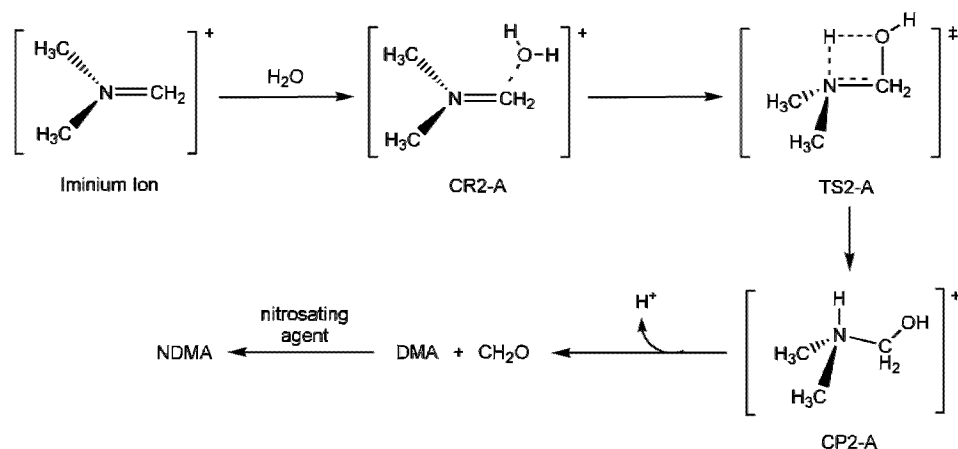
SCHEME 4: Mechanistic Pathway for the Formation of NDMA with the Water-Assisted Hydrolysis Mechanism of Pathway 1
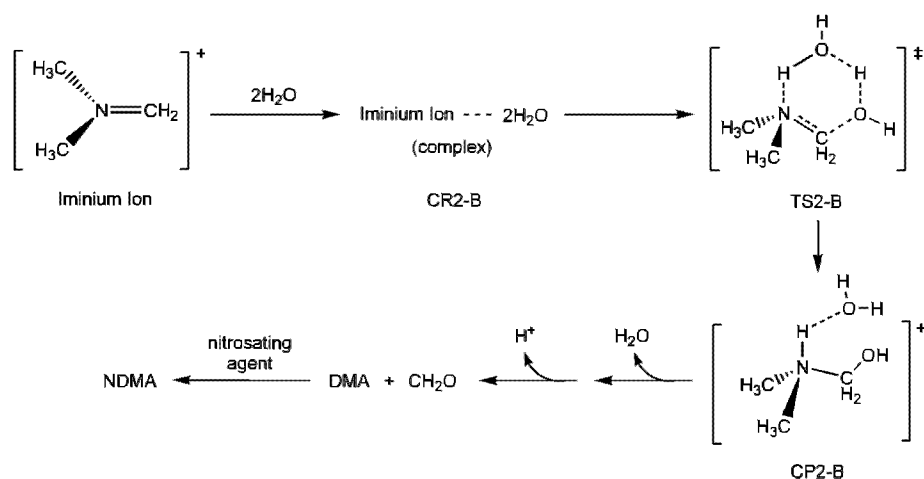
anism (40.21 kcal/mol). This lower energy barrier can be rationalized by the fact that the transition state TS2-B, composed of a six-membered ring, is much more stable than the high-lying four-membered cyclic transition state TS2-A.

In addition to the hydrolysis mechanisms discussed above, the oxygen atom of water molecule may also attack the iminium ion to the nitrogen atom to form two different transition states (TS2-A′ and TS2-B′). These possibilities were also examined; however, the corresponding energy barriers were predicted to be high as 77.78 and 62.93 kcal/mol at the PBE1W/CBSB7 level in the gas phase, respectively. Therefore, they are not expected to occur. The absolute energies were collected in the Supporting Information (Tables S13 and S14).

3.2.2. Reaction Pathway 2: Nucleophilic Attack of NO_2^-. The iminium ion, which is known to be highly reactive toward nucleophiles,[65,66] could therefore be expected to easily undergo the nucleophilic attack by free nitrite (NO_2^-) to give a neutral prereactant (designated as PR3). This prereactant then directly collapses to formaldehyde and NDMA. The detailed reaction pathway is illustrated in Scheme 5, and the fully optimized structures of all stationary points involved in this pathway are collected in Figure 6. The relative energies are listed in Table 4.

As shown in Scheme 5, after the formation of PR3, the movement of electrons may occur to give a transition state (TS3, 383.5i cm^{-1}). It can be found that TS3 is not a real four-
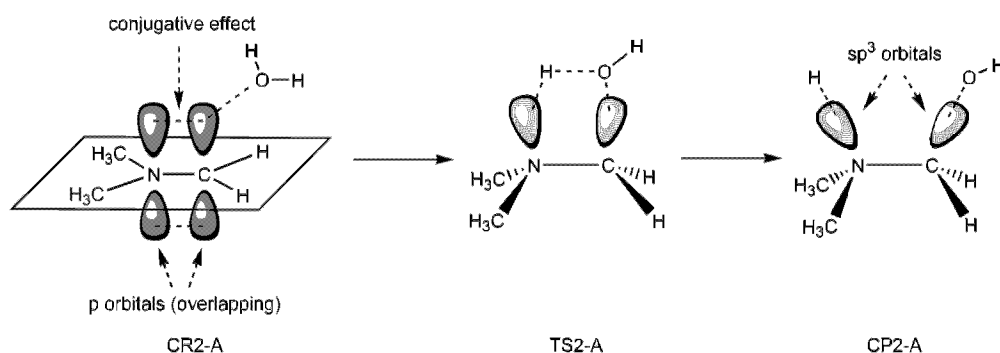

Figure 3. Schematic profile for the conversion of hybridization of N and C atoms involved in the non-assisted hydrolysis mechanism of pathway 1.

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NDMA Formation from Nitrosation of TMA   *J. Phys. Chem. A, Vol. 114, No. 1, 2010* **461**
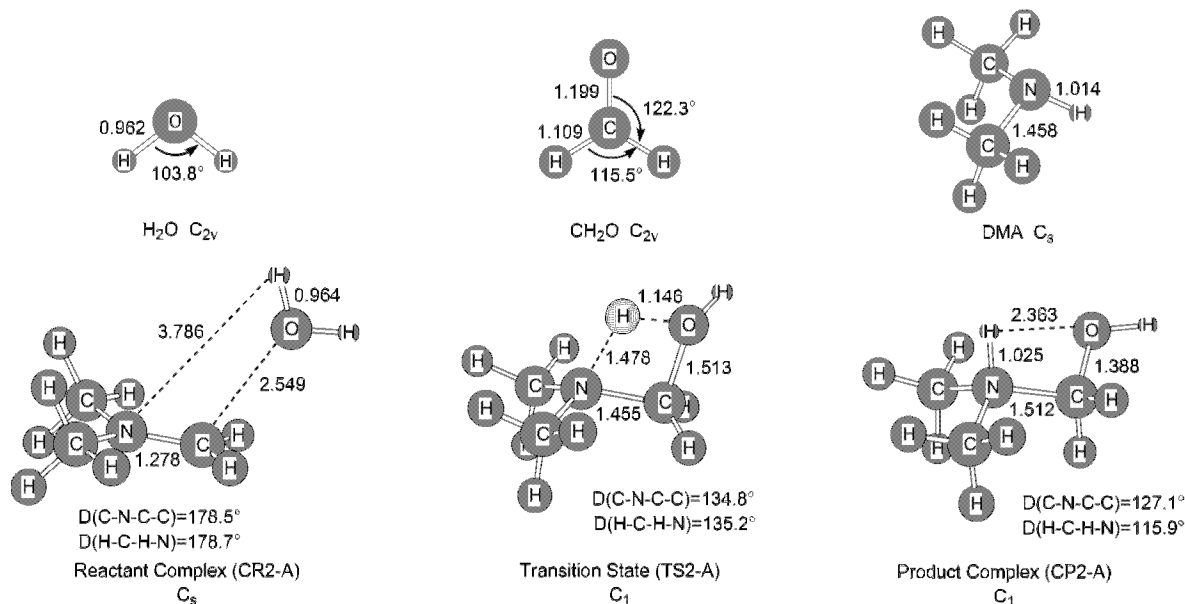
Figure 4. Optimized geometries and main parameters of all the stationary points involved in the non-assisted hydrolysis mechanism of pathway 1 (distances in angstroms, dihedral angle in degrees).
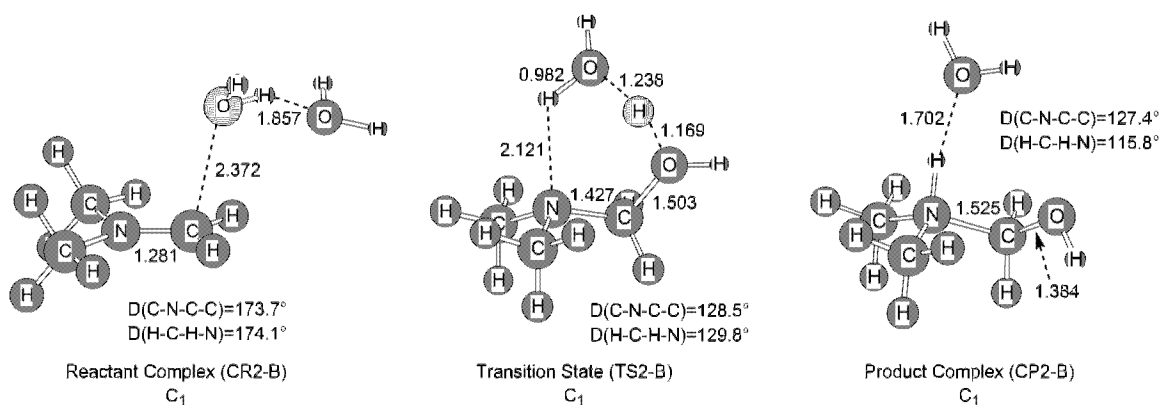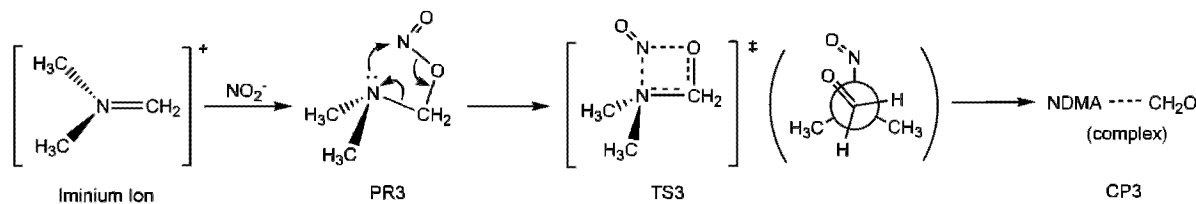

Figure 5. Optimized geometries and main parameters of all the stationary points involved in the water-assisted hydrolysis mechanism of pathway 1 (distances in angstroms, dihedral angle in degrees).

SCHEME 5: Mechanistic Pathway for the Formation of NDMA in Pathway 2

membered cyclic transition state. Newman projection in Scheme 5 implies that TS3 actually holds a conformation between the staggered and eclipsed one, and this is supported by the dihedral angle $D(O-C-N-N)$ calculated to be 45.3°. (See Figure 6.) It can be rationalized by the fact that this conformation avoids strong ring strain and steric hindrance for stabilization. In the process from PR3 to TS3, the changes of bonds shown in Figure 6 indicate that the original $N-C$ and $N-O$ bonds are partially cleavaged, whereas the $N-N$ bond is partially formed. The $C-O$ bond length decreases from 1.430 to 1.257 Å, indicating that a double bond is formed. As shown in Table 4, in the gas phase, the energy barrier for the reaction was calculated to be 27.94 kcal/mol, which is close to that of the water-assisted hydrolysis mechanism in pathway 1 (23.41 kcal/mol).

In the process from TS3 to CP3, two types of geometric changes can be observed: the elongation of the distances of $N-C$

and $N-O$ bonds between CH_2O moiety and the other and the reduction of $N-N$ and $C-O$ bond lengths. In addition, the changes of the two moieties from their previous tetrahedral configuration to a planar configuration demonstrate that both of the N and C atoms change from sp^3 to sp^2 hybridization. Furthermore, changes of the geometry will form the product complex (CP3), in which the formaldehyde and NDMA are connected by two $O-H$ hydrogen bonds, as shown in Figure 6.

3.2.3. Reaction Pathway 3: Direct Nitrosation by N_2O_3. As discussed previously, the optimum pH for the conversion of TMA to NDMA at elevated temperature has been proven experimentally to be about 3.0 to 3.3.[37,38,41,46] It is well known that within this pH range N_2O_3 can be easily formed. On the basis of these results, a new mechanism is proposed in which the iminium ion directly reacts with N_2O_3. The detailed reaction
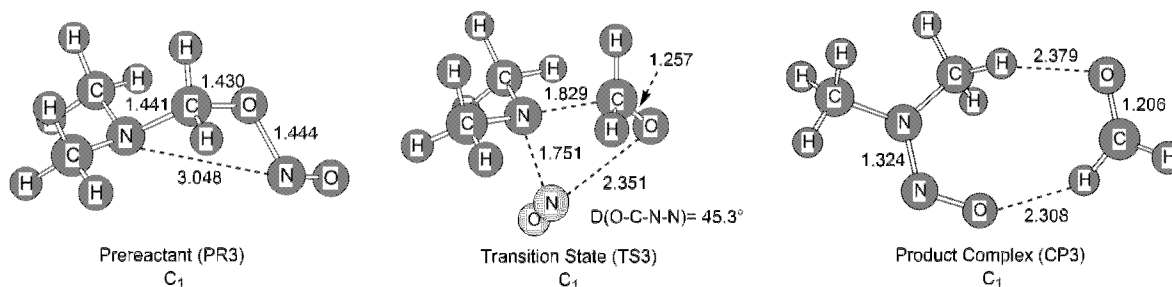
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Figure 6. Optimized geometries and main parameters of all stationary points involved in pathway 2 (distances in angstroms, dihedral angle in degrees).

TABLE 4: Relative Energies (RE), Enthalpies (RH), and Free Energies (RG) of Each Stationary Point Involved in the Reaction of the Iminium Ion with NO_2^- at the CBS-QB3 Level in the Gas Phase and Aqueous Solution[a,b]

species	RE	RH	RG	RE_w[b]
iminium ion + NO_2^-	0.00	0.00	0.00	0.00
PR3	−128.18	−128.74	−117.58	−15.21
TS3	−100.24	−100.72	−89.27	10.25
CP3	−133.31	−132.58	−125.33	−18.95
CH_2O + NDMA	−129.67	−129.13	−130.45	−17.31

[a] Gas phase: relative energies, enthalpies, and free energies in kilocalories per mole. [b] Aqueous solution: relative energies (RE_w) in water at the CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7 level for comparison.

pathway is illustrated in Scheme 6, and the fully optimized structures of all stationary points involved in this pathway are collected in Figure 7. The relative energies are listed in Table 5.

As shown in Scheme 6, two steps are required for this reaction. The first step is the nucleophilic attack of N_2O_3 to the iminium ion to produce a positively charged intermediate (IM), and the second step is the collapse of IM to give the final products: NDMA, formaldehyde, and NO^+. It is evident that NO^+ actually plays the role of catalyst in the whole reaction, and it may reparticipate in the formation of iminium ion. (See Scheme 1.)

The nucleophilic attack of N_2O_3 to the iminium ion starts from a reactant complex CR4, followed by the formation of a five-membered cyclic transition state TS4−1 (345.5i cm^{-1}), which then further produces an intermediate IM. The energy barrier of this step was calculated to be 29.12 kcal/mol in the gas phase, and the enthalpy change ΔH shown in Table 5 indicates that the reaction is an exothermic process. IRC calculation along the reverse reaction pathway shows two types of geometric changes: the elongation of O−C and N−N bond lengths between the iminium ion and N_2O_3 moieties and the reduction of N−N bond length to be N_2O_3 and C−N bond length in iminium ion moiety. These geometric changes indicate that the energy 29.12 kcal/mol required for the transformation from CR4 to TS4−1 is mainly utilized to rotate and dissociate the N−N bond in N_2O_3 as well as elongate the C−N bond in the moiety of iminium ion. During this process, the dihedral angles D(H−C−N−H) and D(C−N−C−C) reduce by 40.8 and 31.9°, respectively. This indicates that the hybridization of the N and C atoms in the iminium ion moiety change from sp^2 to sp^3, which is similar to the case of hydrolysis mechanism (pathway 1, Figure 3). Further changes of the structure encounter an intermediate IM. It can be observed that the N−N bond in the original N_2O_3 moiety is totally separated in IM, and the original planar iminium ion moiety changes into a tetrahedron config-
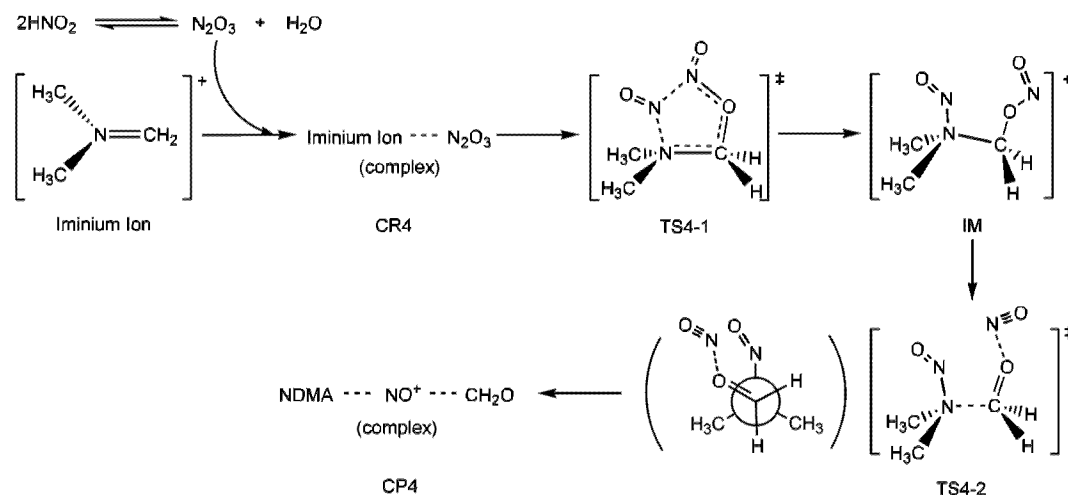
uration as a result of the further reduction of corresponding dihedral angles.

In the second step of the reaction, TS4−2 (211.9i cm^{-1}) connects the nascent intermediate IM and the product complex CP4, as depicted in Scheme 6. The reaction barrier for this step was calculated as 15.31 kcal/mol, which is lower than the barrier (29.12 kcal/mol) of the first step reaction in the gas phase. This implies that the formation of the intermediate IM is the rate-determining step for pathway 3. Notably, the energy barrier (29.12 kcal/mol) of the first step is close to the barrier (23.41 kcal/mol) calculated for the water-assisted hydrolysis mechanism in pathway 1 and the barrier (27.94 kcal/mol) for pathway 2. In addition, it can be found that the products NDMA and formaldehyde have already formed in TS4−2, for which a staggered conformation can be observed. (See the Newman projection in Scheme 6.) The main vibrational mode for the imaginary frequency of TS4−2 corresponds to the stretching vibration of N−C bond, indicating that the energy requirement (15.31 kcal/mol) for overcoming the barrier is mainly utilized to dissociate the N−C bond. Additionally, N−O bond length was also found to be elongated from 1.638 to 1.895 Å when changing from IM to TS4−2. Also, the N−N and C−O bond lengths are decreased by 0.454 and 0.127 Å, respectively, predicting the formation of the two bonds. Further changes of the structure give rise to the product complex (CP4) composed of NDMA, formaldehyde, and NO^+. It is interesting to note that the changes of hybridization of the N and C atoms exhibit a reverse process relative to that of the first step in the reaction (formation of IM from CR4), for both of the two atoms changing back to sp^2 from sp^3 hybridization. This finding is supported by the changes of the corresponding dihedral angles shown in Figure 7.

3.3. Effects of Solvent. Because the attention of this article is focused on environmental nitrosamine formation, for which most reactions should be expected to occur in aqueous solution, the solvent effect of water on NDMA formation from TMA was taken into account. On the basis of the optimized geometries obtained at the CBS-QB3 level, the single-point energy calculation was carried out by the use of the conductorlike polarizable continuum model (CPCM) at the CCSD(T)/6-311+G(d,p) level, denoted as CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7. The corresponding relative energy data (RE_w) in water are presented in Tables 1−5 for comparison with those in the gas phase.

As for the formation of the iminium ion, data in Table 1 show that the energy barrier for the NOH elimination mechanism is 53.62 kcal/mol in aqueous solution. Significantly, the oxidation abstraction mechanism becomes a barrierless process with a negative energy barrier (−9.51 kcal/mol). Moreover, the oxidation abstraction mechanism is exothermic, and a considerable energy (45.24 kcal/mol) was predicted to be released from the

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SCHEME 6: Mechanistic Pathway for the Formation of NDMA in Pathway 3

reaction. Therefore, the TMA$^+$ would be expected to react easily with NO$_2$ because of the low energy barrier. The results actually lead to a conclusion that the oxidation abstraction mechanism is more favored than the NOH elimination mechanism and is probably the one that is mainly operative to form iminium ion. Noticeably, this result supports the feasibility of the experi-

mentally proposed oxidation abstraction mechanism.[63] Moreover, it also indicates that the newly proposed oxidation abstraction mechanism plays an important role not only in the nitrosation of N,N-dialkyl aromatic amines[63] but also in the case of tertiary aliphatic amines. Furthermore, because the formation of N$_2$O$_3$ and its cleavage product NO$_2$ (eqs 4−6) is facilitated
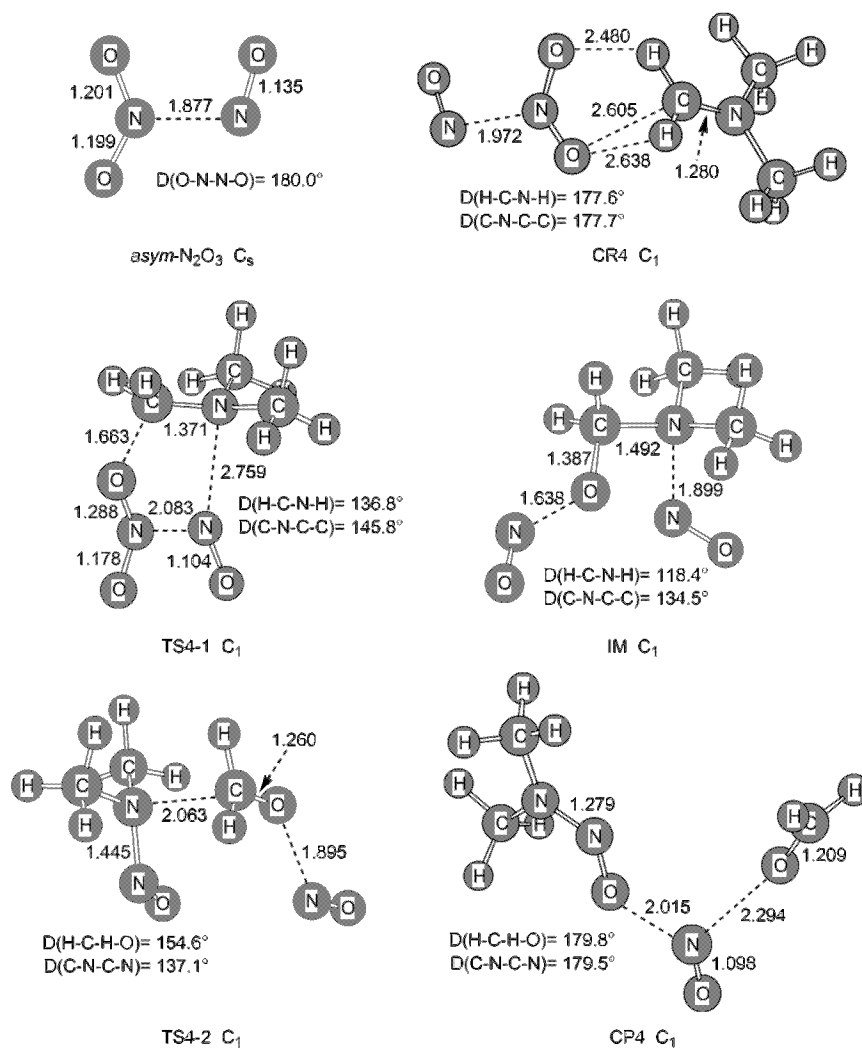

Figure 7. Optimized geometries and main parameters of all stationary points involved in pathway 3 (distances in angstroms, dihedral angle in degrees).

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TABLE 5: Relative Energies (RE), Enthalpies (RH), and Free Energies (RG) of each Stationary Point Involved in the Reaction of the Iminium Ion with N$_2$O$_3$ at the CBS-QB3 level in the Gas Phase and Aqueous Solution[a,b]

species	RE	RH	RG	RE$_w$[b]
iminium ion + N$_2$O$_3$	0.00	0.00	0.00	0.00
CR4	−10.70	−9.97	−2.43	−1.84
TS4−1	18.42	18.16	29.55	15.12
IM	−10.92	−10.89	0.26	−10.57
TS4−2	4.39	4.30	15.87	9.55
CP4	−14.26	−13.21	−5.81	−4.68
CH$_2$O + NDMA + NO$^+$	40.76	42.12	31.13	0.59

[a] Gas phase: relative energies, enthalpies, and free energies in kilocalories per mole. [b] Aqueous solution: relative energies (RE$_w$) in water at the CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7 level for comparison.

in pH 3.0 to 3.3, the results may also explain the experimental fact that the optimum acidity for the nitrosation of TMA within this pH range.[37,38,41,46]

In aqueous solution, the energy barriers for the non-assisted and water-assisted hydrolysis mechanism in pathway 1 were calculated to be 32.84 and 15.30 kcal/mol, respectively, as shown in Tables 2 and 3. The energy barriers obtained at the CPCM-CCSD(T)/6-311+G(d,p)//PBE1W/CBSB7 level are 32.63 and 13.40 kcal/mol, which are close to the data at the CPCM-CCSD(T)/6-311+G(d,p)//B3LYP/CBSB7 level. This indicates that the water-assisted hydrolysis mechanism is still more favored, which is consistent with the case in the gas phase. Accordingly, a conclusion can be drawn that the water-assisted hydrolysis mechanism is more favored than the non-assisted hydrolysis mechanism and should be the one that is mainly operative. The energy barrier for the transformation from PR3 to CP3 in pathway 2 was calculated to be 25.46 kcal/mol (Table 4). With regard to pathway 3, reaction barriers for the first step reaction (formation of the intermediate IM) and the second step reaction (formation of CP4) were calculated to be 16.96 and 20.12 kcal/mol, respectively. This implies that, under experimental conditions, pathway 1 is more feasible than pathways 2 and 3 for the transformation from the iminium ion to NDMA in aqueous solution. Although the new mechanism described in pathway 3, a stepwise reaction of the iminium ion with N$_2$O$_3$, has a relatively higher energy barrier than pathway 1, it is still more favored than pathway 2, a direct reaction of the iminium ion with NO$_2^-$ to form NDMA. Comparing the energy barriers of all steps in the reaction, a conclusion can be drawn that the rate-determining step in aqueous solution is the transformation from the iminium ion to NDMA.

4. Conclusions

The formation mechanisms of NDMA from the nitrosation of TMA were investigated at the CBS-QB3 level of theory. The reaction was proposed to be initiated by the formation of a highly reactive iminium ion, Me$_2$N$^+$=CH$_2$. Two different pathways, that is, oxidation abstraction and NOH elimination mechanisms, were investigated to elucidate the formation of the iminium ion, and the oxidation abstraction mechanism was found to be more favored than the NOH elimination mechanism. The results not only support the feasibility of the experimentally proposed oxidation abstraction mechanism but also extend this mechanism from the nitrosation of *N,N*-dialkyl aromatic amines[63] into the case of tertiary aliphatic amines.

Starting from the iminium ion, three different pathways leading to NDMA were examined. Pathway 1 proposes that the

iminium ion undergoes hydrolysis to give a secondary amine DMA, which then can be directly nitrosated to NDMA. Two different hydrolysis mechanisms were examined for this pathway: non-assisted and water-assisted hydrolysis mechanisms. The energy barrier for the water-assisted hydrolysis mechanism was predicted to be almost half the magnitude of the barrier for the non-assisted mechanism, indicating that the former should be predominant. In pathways 2 and 3, the iminium ion reacts with NO$_2^-$ and N$_2$O$_3$ to form a neutral and a positively charged intermediate, respectively, which then both collapse to NDMA. Comparing the three pathways in aqueous solution, pathway 1 is the most favored, and pathway 3 has a relatively lower energy barrier than pathway 2. All calculation results indicate that the rate-determining step of the whole reaction in aqueous solution is the transformation from the iminium ion to form NDMA.

On the basis of the theoretical study reported in this article, some experimental results on the *N*-nitrosamines formation from tertiary amine can be explained. This work will be helpful to elucidate the formation mechanisms of *N*-nitrosamines from tertiary amines.

Supporting Information Available: Absolute energy for each stationary point involved in the formation of NDMA from the nitrosation of trimethylamine. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Exhibit 44

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2013 WL 3466821
Only the Westlaw citation is currently available.
NOT FOR PUBLICATION
United States District Court, D. New Jersey.

In re FRONT LOADING WASHING
MACHINE CLASS ACTION LITIGATION.

Civil Action No. 08–51(FSH).
|
July 10, 2013.

OPINION

HOCHBERG, District Judge.

*1 This matter comes before the Court upon Plaintiffs'
motion for class certification.[1] Defendant opposes class
certification and moves to strike evidence relied upon by
Plaintiffs in support of its Motion for Class Certification
as inadmissible. Defendant has also filed a *Daubert* motion
to exclude expert testimony. Plaintiffs have also filed a
Daubert motion to exclude testimony from Defendant's
expert witnesses.

I. Background

Plaintiffs bring the instant action on behalf of themselves and
a nationwide class of persons who purchased front-loading
automatic washing machines that were marketed and sold by
LG, and that allegedly have a common drainage defect that
causes the proliferation of mold and mildew, as well as foul
odors in the machines and on clothing washed in the machine.
More specifically, Plaintiffs allege that all of LG's front load
washing machines ("LG FLW" or "FLW") have a common
design defect in the dryer drum and/or door gasket that causes
water to not fully or properly drain after each wash cycle,
and that this remaining water causes the proliferation of mold
and mildew. Plaintiffs assert claims against Defendant under
the New Jersey Consumer Fraud Act, ⚑N.J.S.A. § 56:8–1,
et seq., Magnuson–Moss Warranty Act, 15 U.S.C. § 2301,
et seq., for breach of express and implied warranties under
New Jersey law, and for unjust enrichment under New Jersey
law. In the alternative, Plaintiffs bring claims for breaches
of express and implied warranties, unjust enrichment and
consumer fraud and deception under the laws of the states
where Plaintiffs reside.

Plaintiffs have moved to certify a nation-wide class of
those who purchased LG FLW in the United States and, in
the alternative, have moved to certify classes to apply the
substantive law of the state where the Plaintiffs reside and/
or purchased their LG FLW. Plaintiffs move to apply New
Jersey law to the entire class if certified as a whole, arguing
that Defendant's principal place of business is in New Jersey,
many members of the class reside in New Jersey, Defendant's
alleged misconduct occurred in New Jersey and New Jersey
has a significant interest in seeing this matter litigated within
the state.

II. DISCUSSION

A. Daubert Motions

1. *Legal Standard*

Federal Rule of Evidence 702 provides the relevant legal
standard in a *Daubert* motion. Pursuant to Fed.R.Evid. 702,

> If scientific, technical, or other
> specialized knowledge will assist the
> trier of fact to understand the evidence
> or to determine a fact in issue, a
> witness qualified as an expert by
> knowledge, skill, experience, training,
> or education, may testify thereto in the
> form of an opinion or otherwise, if (1)
> the testimony is based upon sufficient
> facts or data, (2) the testimony is
> the product of reliable principles and
> methods, and (3) the witness has
> applied the principles and methods
> reliably to the facts of the case.

Fed.R.Evid. 702. The United States Court of Appeals for the
Third Circuit has determined that Rule 702 "has a liberal
policy of admissibility."[2]

*2 Two United States Supreme Court cases, ⚑*Daubert v.*
Merrell Dow Pharms., Inc., 509 U.S. 579, 113 S.Ct. 2786, 125
L.Ed.2d 469 (1993) and ⚑*Kumho Tire Co. v. Carmichael,*
526 U.S. 137, 119 S.Ct. 1167, 143 L.Ed.2d 238 (1999), have
further defined the legal standard for expert testimony. The
goals of the *Daubert* standard, as established by the Supreme

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Court, are to determine: 1) whether the expert utilized the scientific method and whether the expert's testimony is based on scientifically valid principles; and 2) whether the expert testimony is relevant to the proposing party's argument. [3]

In *Kumho,* the Court expanded the scope of the *Daubert* standard, explaining that while the Court in *Daubert* directly addressed scientific testimony, the resulting standard was not intended to apply exclusively to scientific testimony. Instead, *Kumho* established that the *Daubert* standard is appropriate for all experts providing specialized knowledge and held that "[t]he trial judge's effort to assure that the specialized testimony is reliable and relevant can help the jury evaluate that foreign experience, whether the testimony reflects scientific, technical, or other specialized knowledge." [4] This is true even if the experts are providing experience-based testimony. [5]

To determine if the testimony is reliable, the Third Circuit has established an eight-part test which this Court must consider:

(1) whether a method consists of a testable hypothesis;

(2) whether the method has been subject to peer review;

(3) the known or potential rate of error;

(4) the existence and maintenance of standards controlling the techniques operation;

(5) whether the method is generally accepted; [6]

(6) the relationship of the technique to methods which have been established to be reliable;

(7) the qualifications of the expert witness testifying based on the methodology; and

(8) the non judicial uses to which the method has been put. [7]

⚑ *In re Paoli Railroad Yard PCB Litigation,* 35 F.3d 717, 742 (3d Cir.1994). This test may be more flexibly applied in cases where the expert testimony is based on experience. [8]

As explained by the Court in *Kumho,* the expert's testimony must be related to the case at hand as well. According to this Court, "[t]he role of the judge with regard to expert testimony is to serve as a gatekeeper, and it is the judge who must decide

if an expert's testimony reliably "fits" the case." [9] The party proffering the expert will have the burden to demonstrate that the testimony is admissible, on a preponderance of the evidence standard . [10]

2. Defendant's Pending *Daubert* Motion to Exclude Inadmissible Expert Testimony

Plaintiffs have proffered two witnesses: Dr. R. Gary Wilson ("Wilson") and Dr. Chin Yang ("Yang"). Defendant contends that this Court should exclude the opinions of Wilson and Yang because both allegedly lack a reliable methodology and opine in areas where neither is qualified to serve as an expert witness. [11]

a. Dr. R. Gary Wilson

*3 Plaintiffs offer Dr. Wilson's testimony in support of class certification to establish that: (1) every LG FLW, regardless of model, year, or platform, shares a materially uniform design because all of them "provide the perfect environment for mold and mildew ... a humid environment with little ventilation;" and (2) every LG FLW, regardless of model, year, or platform, fails to self clean, which leads to biofilm accumulation that results in foul odors. [12] Wilson Supp. Rep. at 4, 5–6. Dr. Wilson's opinions are intended to demonstrate commonality and predominance [13] in support of Plaintiffs' class certification motion. According to Plaintiffs, the testimony provided by Wilson is mainly experience-based, supplemented by research conducted by Wilson for this litigation.

Defendant argues that Wilson has never designed a front-load washing machine and that before he was retained by Plaintiffs, he had no experience with LG FLWs. Defendant argues that because of his lack of previous experience, Wilson's opinions about LG FLWs are based solely on the work he did in this case. LG claims that Wilson did almost no work in this case because he never tested any LG FLW and inspected just a few LG FLWs. Of the 19 LG FLWs owned by Plaintiffs, Defendant claims that Wilson disassembled and inspected just one and also inspected three other LG FLWs provided to him by Plaintiffs' counsel. Accordingly, Defendant argues that Wilson's opinions are admissible only if based on testing or some other reliable methodology, and that Dr. Wilson has none.

Dr. Wilson has a Ph.D. in Mechanical Engineering from Case Western Reserve University and a Masters Degree in

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Mechanical Engineering from the University of Illinois. [14] Wilson is also a Lecturer in the Mechanical Engineering & Engineering Sciences Department at the University of North Carolina. Dr. Wilson worked for Whirlpool for 23years, working with both top-loading washing machines and front-loading machines. When he retired from Whirlpool he was the Director of Laundry Technology.

While there does not appear to be a dispute about Wilson's knowledge of mechanical engineering, the parties disagree about the characterization of Wilson's experience. According to Plaintiffs, Wilson has two decades of experience with designing washing machines but Defendant argues that Wilson has never actually designed a front-loading washer and has spent a majority of his career working on refrigerators, air-conditioners and dishwashers. Defendant further argues that Wilson only worked on Whirlpool washing machines from 1985–1988, and even then the work was not on FLW, making the experience irrelevant.

While Defendant claims Wilson has limited practical work experience with washing machines, it is apparent from some of Defendant's submissions, as well as Dr. Wilson's own characterization of his experience, that he was the director of laundry technology at Whirlpool from 1997–99.Wilson was employed by Whirlpool from 1976 to 1999.

*4 After considering the testimony rendered at the *Daubert* hearing and the record in this matter, the Court will permit the expert testimony of Dr. Wilson. The Court agrees with the Court in *Butler v. Sears,* No. 06–7023 (N.D.Ill. September 30, 2011), which found on a *Daubert* challenge of Dr. Wilson that:

> The value of Wilson's testimony is not based upon his sampling methods; it is instead based upon his knowledge of washer technology and his understanding of the principles that generally keep machines functionally clean, as well as the extent to which the subject machines depart from those principles. In the court's view, Wilson is clearly qualified to use his knowledge of those principles to offer an opinion, for purposes of a class certification motion, that all front loading high efficiency machines are similarly defective in design. The fact

that his opinion does not account for mitigating model changes that do not alter the machines' basic design is relevant to the weight to be assigned to his opinion, but does not indicate that the opinion is inadmissible in support of the certification motion.

Id. at 6–7. Dr. Wilson's opinion is based on the extensive experience he has gained during his more than 20 years as an engineer with Whirlpool, his education, his training, his technical expertise, as well as his review and analysis of the FLWs in this and related litigation. The fact that Dr. Wilson has not undertaken his own testing does not disqualify him as an expert for the purposes for which he is proffered.

Wilson is qualified to testify regarding Plaintiffs' theory of the case and Plaintiffs are entitled to attempt to prove their theory of the case, *i.e.,* that Defendant's washing machines contain a common design defect. [15] Accordingly, the Court is satisfied that Dr. Wilson has satisfied Fed.R.Evid. 702 and his expert report will be permitted for purposes of the Motion for Class Certification.

b. Dr. Chin S. Yang

Dr. Yang is a PhD microbiologist who specializes in mycology, the study of fungi, and he has expertise in the identification and growth of fungi. He is employed as the Scientific & Technical Advisor and Senior Consulting Scientist at Prestige EnviroMicrobiology, Inc., a company that specializes in analyzing samples for fungi, mold and bacteria. He has testified regarding the environments inside FLW and microbiology issues associated with FLW in at least three other cases.

Plaintiffs offer Dr. Yang's testimony for the proposition that LG's FLW "provide an ideal environment for Biofilm growth" in part because they contain "spaces for trapping moisture and nutrients" and for the proposition that "[t]he trend of using a lower washing machine temperature increases the risk of microbes surviving laundering." Dr. Yang is offered primarily as a rebuttal expert. [16] He analyzed the "mold and fungi testing data collected by LG's experts and concluded that the testing was not performed in conformity with the lab's standard operating procedure, [and] that the testing did not include tests for bacteria, a major odor-causing component of biofilm." His testimony also would support Dr. Wilson's and

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Plaintiffs' theory of the case, *i.e.,* that a common design defect has caused the proliferation of mold and mildew. Defendant maintains that Dr. Yang's opinions fail the reliability test of *Daubert* because Dr. Yang hasn't conducted any testing, and in fact, hasn't done anything but look at photographs.

*5 After considering the record, the Court will permit the testimony of Dr. Yang. Dr. Yang's opinions are based on his education and experience as a mycologist, as well as the significant number of articles he has researched and written in this field and his analytic experience. Dr. Yang can rely on the testing done by other experts and opine as to whether their methods of testing would mimic the growth of mold in the real world. As a microbiologist who specializes in mycology, he certainly is qualified to look at photographs and opine on whether what he is looking at is biofilm, mold, mildew, fungi or bacteria. He is qualified to opine with respect to how mold grows, the types of environments that mold typically grows in and whether it might exist even if you cannot see it. Dr. Yang does not need to have conducted his own testing to rebut Dr. Caulfield's opinion that the machines he tested had no mold in them and that he had done his testing in a way that mimicked the way Plaintiffs should have been using their LG FLWs. Accordingly, Defendant's *Daubert* challenge to Dr. Yang is denied and he will be permitted to rebut the expert testimony of Dr. Caulfield for class certification purposes.

3. Plaintiffs' Pending *Daubert* Motion to Exclude Inadmissible Expert Testimony

Defendant has proffered three witnesses: Dr. Charles J. Wysocki ("Wysocki"), Dr. Edward M. Caulfield ("Caulfield") and Dr. Thomas Maronick ("Maronick"). Plaintiffs argue that the Court should exclude all three opinions, contending that Wysocki's opinion fails the "fit" aspect of the *Daubert* test, and that both Caulfield's and Maronick's opinions fail to fit and are unreliable.

a. Dr. Charles J. Wysocki

Defendant offers Dr. Wysocki for the purpose of helping the Court determine whether it is proper to assume that every LG FLW owner experienced an intense bad smell from the machine, and explain why it is improper to reach such a conclusion. Dr. Wysocki opines: (1) that each person's olfactory abilities and experiences are different; (2) whether a person perceives an odor, whether it is perceived as pleasant or unpleasant, and whether it is perceived intense, depends on a variety of factors that vary from person to person; and (3)

it is therefore not possible to generalize about the olfactory experiences of all LG FLW owners.

According to Defendant, Wysocki is "one of the world's leading experts on individual difference in olfaction." A member of Monell Chemical Senses Center, the only independent, non-profit scientific institute dedicated to the research of smell and taste, Wysocki has his Ph.D. in psychobiology from Florida State University and has also been a teacher at the University of Pennsylvania. He has spent decades studying individual differences in olfaction. The National Institutes of Health have supported his research into individual differences in odor perception for over 25 years.

Plaintiffs do not contend that Dr. Wysocki lacks the education or experience to testify as an expert witness for Defendant. Instead, Plaintiffs contend that Defendant presents Dr. Wysocki as an expert on the basis of the scientific tests he conducted for this proceeding and that Dr. Wysocki should be excluded because these scientific tests fail to meet the *Daubert* criteria.

*6 According to Plaintiffs, there are five reasons why Dr. Wysocki should not be allowed to testify as to the Rule 23 criteria Plaintiffs must establish to certify their class, including:

1. There is no linkage or fit between Dr. Wysocki's opinion and the facts of the case;

2. Dr. Wysocki makes no effort to relate his knowledge to the facts;

3. Dr. Wysocki admits having no idea whether his opinions would be helpful to the case;

4. Dr. Wysocki has not tested the Plaintiffs' ability to smell; and

5. Dr. Wysocki has not tested the FLW to see what odorants existed.

Defendant argues that Dr. Wysocki was not required to show these things, as he was there to offer his opinions about olfactory abilities and experience among all people generally. Essentially, Dr. Wysocki supports Defendant's argument that there can never be a class certified on the basis of odors, as there is too much variation among people's abilities to smell.

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The Court will permit the testimony of Defendant's olfaction expert, Dr. Wysocki. He is proffered by Defendant in an effort to attempt to defeat Plaintiffs' arguments regarding commonality and typicality. The Court accepts Defendant's proposition, via Dr. Wysocki, that people smell things differently. For many of the same reasons that the Court will permit Dr. Wilson for Plaintiffs, the Court will permit Dr. Wysocki for Defendant.

Clearly, the parties have divergent theories of the case. Plaintiffs believe that LG's FLWs have a common design defect and Defendant believes that because everyone has a different sense of smell, the commonality prong of class certification cannot be met. The Court will honor each party's right to demonstrate its theory of the case through its expert that supports its theory. From the record before the Court as well as the *Daubert* hearing, it is evident that Dr. Wysocki has the educational and practical expertise to opine that the class cannot satisfy the commonality prong because different people smell different things differently. The Court finds that there is a fit between Dr. Wysocki's opinion and the facts of this case and he has satisfied the *Daubert* analysis.

b. Dr. Thomas Maronick

Defendant offers Dr. Maronick to challenge Plaintiffs' assertion of predominance in support of their class certification motion. To assess whether the problems asserted by Plaintiffs were widespread, LG retained Dr. Maronick to conduct an internet-based consumer survey to determine the incidence of problems among the population of LG FLW owners and the overall satisfaction or dissatisfaction with the machines. Plaintiffs argue that Dr. Maronick's survey evidence should be excluded because it is not the result of any scientific or otherwise reliable testing. Plaintiffs also challenge his methodology. Plaintiffs assert that while he conducted an internet-based opinion poll, no controls were put in place to ensure that respondents were representative of anything. Therefore, Plaintiffs assert that Dr. Maronick's survey is not reliable and should therefore be excluded under *Daubert.*

 *7 Dr. Maronick is a Professor of Marketing at Towson University in Maryland, where he teaches "the proper methods and procedures for designing and implementing consumer surveys, including internet-based surveys," and is the Director of Impact Evaluation in the Bureau of Consumer Protection at the FTC where he has gained experience designing and implementing over 300 marketing and consumer surveys. Dr. Maronick has written numerous articles relating to consumer and internet surveys and has frequently qualified as an expert witness in litigation.

The Court will not permit the testimony of Dr. Maronick because it is just too unreliable to be of help in deciding class certification. Dr. Maronick only asked highly general questions. For example, He never asked "does your machine smell?" or any other similar question of that nature designed to elicit a response that would have indicated whether or not the survey responders had the same mold and odor issues as Plaintiffs. Dr. Maronick also cannot say much of anything about who answered his internet survey. It is unclear whether the people who took the survey were paid or otherwise rewarded to take it. There is no way of knowing how many people Dr. Maronick surveyed. In his survey results, he included identical answers to questions even when they were open-ended because he had no way of knowing if they were from different individuals. Dr. Maronick can't say for sure whether any survey-takers actually owned LG FLWs. Identifying data was not requested, such as serial number or other criteria tending to establish that the survey responder really owned the product. Defendant is taking an inferential leap by arguing that this survey leads to an inference that the survey-takers never smelled any mold in their machines just because they indicated during the survey that they were happy with their machines, without any specific query about smell, when that could easily have been asked. For all of these reasons, the Court finds that Dr. Maronick's opinion does not satisfy Fed.R.Evid. 702. His opinion is not helpful in deciding class certification.

c. Dr. Edward M. Caulfield

Defendant proffers Dr. Caulfield for class certification purposes to show there is no uniform design defect in the machines, but rather, that the improper use and maintenance of Plaintiffs' washing machines caused the buildup and odors. He opines that owners who follow instructions will not have them. [17] Dr. Caulfield is a mechanical engineer with a Ph.D. in theoretical and applied mathematics, is a registered Professional Engineer in two states, taught at a university level, and has decades of engineering work experience which includes design reviews, evaluations, and testing.

Plaintiffs contend that Dr. Caulfield's testing does not fit the issues of the case because it does not accurately reflect ordinary use of the LG FLWs. Plaintiffs also maintain that Caulfield's test is unreliable because it does not test what it purports to test. For example, Caulfield offers no objective

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criteria to support his premise that, when testing whether mold and/or smells develop in the FLW, 15 loads per week for 24 weeks is equivalent to 7.5 loads per week for 52 weeks (the same total number of loads that an average household runs in a year).

*8 The arguments made by Plaintiffs are generally substantive disagreements with the timing choices in his testing process, which can be tested on cross examination. They do not disqualify him as an expert. Although Caulfield chose to perform a test, rather than rely on his extensive knowledge, experience and education, his tests are relevant to the matter at hand. The Court recognizes that Dr. Caulfield changed the typical actual usage of washing machines by shortening the span of usage time. This is a factor that goes to the weight the Court will afford Dr. Caulfield's opinion, and not to its admissibility. The Court will consider Dr. Caulfield's testimony for what it is worth. Although Dr. Caulfield's testimony might be stronger had he actually ran his test over a year-long period, doing 7.5 loads per week like the average household, this factor goes to weight and not admissibility. Accordingly, the Court finds that Dr. Caulfield's opinion could be helpful to the Court in determining whether class certification is appropriate in this action.[18]

B. Pending Motion to Strike Inadmissible Evidence in Plaintiffs' Motion for Class Certification

Defendant has moved to strike alleged inadmissible evidence included within Plaintiffs' Motion for Class Certification. This alleged inadmissible evidence can be grouped into five separate categories. These groups consist of: 1) evidence already stricken from the case; 2) declarations Plaintiffs submit in support of their motion that contradict sworn deposition testimony; 3) subsequent remedial design changes presented to prove a design defect; 4) inadmissible hearsay statements made by other companies; and 5) incomplete, uncertified translations.

1. Evidence Already Stricken From This Case

Defendant argues that Plaintiffs have included three expert reports within their motion for class certification which make references to declarations that have already been deemed inadmissible by Judge Shipp because they were disclosed by Plaintiffs after the close of discovery. Plaintiffs concede that references to the declarations in the reports were inadvertently included and will be removed. However, this issue does not end here as Defendant asks the Court to strike all of Plaintiffs' expert reports due to the references to the declarations.

It does not appear that Plaintiffs acted in bad faith in including this evidence; rather, it was simply a matter of inadvertence. Therefore, the Court will not strike the entirety of the expert reports. However, Plaintiffs are cautioned that when they re-file their motion for class certification, they cannot rely on evidence previously stricken from the case; Plaintiffs, as well as their experts, must comply with Court orders. Accordingly, Defendant's motion to strike the full reports is denied.

2. Sworn Deposition Testimony and Declarations

Defendant argues that in support of their motion for class certification, every Plaintiff but one has submitted a declaration and that most of these declarations contradict Plaintiffs' deposition testimony. The statements within the declarations giving rise to the instant motion pertain to: purchase as a result of advertisement, the date at which Plaintiffs first noticed mold and odor, and review of the original complaint. Plaintiffs respond to Defendant's claims by arguing that the declarations are consistent and are really just clarifications. Assuming arguendo that the declarations were inconsistent, Plaintiffs argue that the "sham affidavit doctrine" is not applicable here.

*9 According to the United States Court of Appeals for the Third Circuit, the sham affidavit doctrine states that "a party may not create a material issue of fact to defeat summary judgment by filing an affidavit disputing his or her own sworn testimony without demonstrating a plausible explanation for the conflict." *Baer v. Chase,* 392 F.3d 609, 624 (3d Cir.2004).

This Court is not currently facing a summary judgment motion, a clear aspect of the sham affidavit rule as explained by the Third Circuit in *Baer,* but rather a motion to certify a class. Therefore, a sham affidavit doctrine argument is not currently appropriate and does not require the Court to disregard the Plaintiffs' disputed declarations.

Moreover, the inconsistencies that do exist do not rise to the level of sham pleadings. *See Cottrell v. Zagami, LLC,* 2010 U.S. Dist. LEXIS 63371, 2010 WL 2652229 (D.N.J. June 23, 2010). In *Cottrell,* the defendants argued that even if the plaintiff was not subject to the sham affidavit doctrine because the case was not yet at the summary judgment stage, the court should have refused to accept sham pleadings. *Id.* at * 19. Because the inconsistencies were "not so outlandish as to be facially false" nor were they indicative of gamesmanship, the

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Cottrell court found it could not declare that the plaintiff had submitted sham pleadings. *Id.* at * 19–20.

Here, the inconsistencies complained of by Defendant seem to pertain to differences in interpretation. Defendant finds the phrase "advertised as an efficient appliance," to mean all Plaintiffs viewed an advertisement, while Plaintiffs intended the phrase to mean that Plaintiffs were aware at some point in the buying process that LG marketed the appliances as efficient. In the same vein, Defendant argues that all Plaintiffs did not view the original complaint, as some joined after the complaint had been amended, while Plaintiffs argue they intended to convey that all Plaintiffs viewed *their* original complaint, where the amended complaint was the original complaint for some Plaintiffs. Finally, the issues with the dates appear to be mainly clerical errors.

The Court finds that the small differences between the declarations and the depositions, if any, do not appear to be facially false as to be sham pleadings. Accordingly, Defendant's motion to strike Plaintiffs' declarations pursuant to the sham affidavit doctrine is denied. However, Plaintiffs are directed to correct the clerical errors with respect to the dates. The Court notes that there was really no objective basis for invoking these doctrines screaming "sham" and asks both sides to tone down the rhetoric and file only motions that are genuinely based on the circumstances. Any further waste of court time on groundless motions will risk sanctions, and all parties are now on notice.

3. Remedial Design Defects

Federal Rule of Evidence 407 states that:
When measures are taken that would have made an earlier injury or harm less likely to occur, evidence of the subsequent measures is not admissible to prove:

> ***10** • negligence;
>
> • culpable conduct;
>
> • a defect in a product or its design; or
>
> • a need for a warning or instruction.

But the court may admit this evidence for another purpose, such as impeachment or—if disputed—proving ownership, control or the feasibility of precautionary measures.

The Court is well aware of what is, and is not, permitted by Rule 407 and will be mindful of the appropriate purposes when ruling on the class certification motion. Plaintiffs will be permitted at trial to introduce evidence of remedial design defects only in a manner consistent with Rule 407, *i.e.,* Plaintiffs will not be able to use the evidence for any purpose barred by Rule 407 and can introduce the evidence for purposes consistent with the last sentence of Rule 407, with a proper limiting instruction. These rulings will be made at trial.

4. Hearsay Statements

Defendant argues that the motion for class certification impermissibly relies on inadmissible hearsay statements made by other companies. Defendant relies on one case, *Agere Sys. v. Adv. Enviro Tech.,* 602 F.3d 204, 232 (3d Cir.2010), in support of this proposition. However, as Plaintiffs aptly point out, *Agere* did not involve class certification and did not hold that documents containing hearsay should be stricken during class certification.

The Federal Rules of Evidence are not stringently applied during class certification and courts may consider evidence that might later be ruled inadmissible at trial. *See, e.g.,* *Eisen v. Carlisle & Jacquelin,* 417 U.S. 156, 178, 94 S.Ct. 2140, 40 L.Ed.2d 732 (1974) (court's determination of class certification is based upon "tentative findings, made in the absence of established safeguards" and explaining that class certification is "of necessity ... not accompanied by the traditional rules and procedures applicable to civil trials"); *Lujan v. Cabana Mgmt., Inc.,* No. 10–755, 2011 WL 317984, *4 (E.D.N.Y. Feb.1, 2011); ("courts frequently consider hearsay in deciding whether to issue class notice"); *Hayden v. Freightcar Am., Inc.,* No. 07–201, 2008 WL 375762, at *2 (W.D.Pa. Jan.11, 2008) (evidence proffered in support of a motion to certify a class need not be admissible at trial); *Vinson v. Seven Seventeen HB Phila. Corp.,* No. 00–6334, 2001 WL 1774073, at *20 n. 28 (E.D.Pa.Oct.31, 2001) ("On a motion for class certification, the evidentiary rules are not strictly applied and courts will consider evidence that may not be admissible at trial."); *In re Hartford Sales Practices Litig.,* 192 F.R.D. 592, 597 (D.Minn.1999) ("On a motion for class certification, the evidentiary rules are not strictly applied and courts will consider evidence that may not be admissible at trial."). In addition, at the class certification stage, courts often examine pleadings as well as affidavits, containing hearsay. *See Eisenberg v. Gagnon,* 766 F.2d

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770, 786 (3d Cir.1985); *Roe v. Operation Rescue,* 123 F.R.D. 500, 502 (E.D.Pa.1988).

*11 Plaintiffs may rely on the challenged documents which may or may not contain hearsay in support of their class certification motion. The Court recalls that at least some of the documents complained of were produced by parent or affiliate companies of LG and as such, may be admissions and not constitute hearsay at all. The Court notes that it is rather hard for LG to argue that documents from its parent company are inherently unreliable.

5. Translations

Defendant argues that Plaintiffs' motion papers have taken out of context and mischaracterized statements in all of the Korean-language documents. Defendant essentially maintains that Plaintiffs failed to comply with the Federal Rules of Evidence because: (1) Plaintiffs often attach to their motion only selected pages of a document, rather than the complete document in violation of Fed.R.Evid. 106's rule of completeness; (2) Plaintiffs never translate the entire document to provide its complete context and content also in violation of Fed.R.Evid. 106's rule of completeness; and (3) Plaintiffs do not provide any certified translations in violation of Fed.R.Evid. 604.

A party may provide the Court with excerpted portions of testimony and evidence in support of its motion and LG is free to provide the Court with the full exhibits to provide context of the documents and corrected translations if it wishes to do so. For key disputes about translation, certified translations will be required.

To save time, the Court asks the parties to meet and confer and provide the Court with a joint translation and joint set of the relevant portions of the documents sought by both sides by July 16, 2013. If agreement cannot be reached, the Court will refer the parties to a Special Master and will cost-shift if it determines that either side acted inflexibly or unreasonably.

III. CONCLUSION

For the reasons stated above, Defendant's motion to exclude Plaintiffs' experts Dr. Wilson and Dr. Yang is denied. Plaintiffs' motion to exclude Defendant's experts Dr. Caulfield and Dr. Wysocki also is denied. However, Plaintiffs' motion to exclude Defendant's expert Dr. Maronick is granted. Defendant's motion to strike inadmissible evidence is denied. An appropriate Order shall issue.

All Citations

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Footnotes

1   The Court administratively terminated Plaintiffs' Motion for Class Certification on July 7, 2011 so that it could rule on the three other pending motions and then determine whether new briefing on the Motion for Class Certification was required.

2   ⚑*Pineda v. Ford Motor Co.,* 520 F.3d 237, 243 (3d Cir.2008).

3   ⚑*Daubert,* 509 U.S. at 580.

4   ⚑*Kumho Tire Co.,* 526 U.S. at 149.

5   *Id.*

6   The Supreme Court determined, however, that the expert's theory or technique was not required to have "general acceptance" throughout the relevant scientific community. ⚑*Daubert,* 509 U.S. at 597.

7   ⚑*U.S. v. Mitchell,* 365 F.3d 215, 235 (3d Cir.2004).

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8   ⚑*Kumho,* 526 U.S. at 141–42.

9   *U.S. v. Schiff,* 538 F.Supp.2d 818, 834 (D.N.J.2008) (citing ⚑*In re Paoli R.R. Yard PCB Litigation,* 35 F.3d at 748 ("*Daubert* makes clear for the first time at the Supreme Court level that courts have to play a gatekeeping role with regard to experts")).

10   ⚑*Daubert,* 509 U.S. 579, 113 S.Ct. 2786, 125 L.Ed.2d 469.

11   Specifically, Defendant argues: "Because Wilson and Yang did no work and no testing and instead looked at just a few LG FLWs hand-picked by Plaintiffs' counsel, their opinions flunk the reliability requirement under Federal Rule of Evidence 702 and *Daubert.* Their opinions are in fact only testable hypotheses, but they did no testing and applied no other reliable methodology." Brief in Support of Defendant LG USA's *Daubert* Motion at 2.

12   The Rule 23 class factors he is being offered to support are commonality and predominance.

13   To the extent that commonality and predominance overlap with typicality, Dr. Wilson's opinions also are offered in support of that.

14   Plaintiff's Exhibit 11: Dr. Wilson's Expert Report, 15.

15   Many of Defendant's arguments in support of its *Daubert* motion to exclude the testimony of Dr. Wilson appear to relate to the credibility of Wilson as an expert, a question of fact that is left to a fact-finder, rather than Dr. Wilson's admissibility. Defendant's disagreement with Plaintiffs' theory of the case and with Dr. Wilson's testimony and report goes to the weight the Court will give the expert testimony and not to whether Dr. Wilson is himself qualified to give his expert opinion on Plaintiffs' theory of the case and the LG FLWs.

16   For example, Dr. Yang is introduced to rebut the expert opinion of Dr. Caulfield, one of LG's experts. Dr. Caulfield ran certain tests where he accelerated the rate at which laundry was done in an effort to mimic the way Plaintiffs were using their machines and in an attempt to demonstrate that doing laundry the "right" way would not cause the proliferation of mold. Dr. Yang is introduced by Plaintiffs to opine that accelerated testing cannot accelerate mold growth because it takes a certain amount of time for mold to grow.

17   Defendants maintain that use is therefore an individual factor for every member of the class. Additionally, they argue that this also relates to typicality.

18   Because Dr. Caulfield admits he doesn't know if the machines contained mold or not he will not be able to opine as to that.

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